Effect of fungicides on Fusarium ear blight and mycotoxin accumulation in winter wheat (*Triticum aestivum* L.)

Thesis

UNRESTRICTED

EFFECT OF FUNGICIDES ON FUSARIUM EAR BLIGHT AND MYCOTOXIN ACCUMULATION IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

STOYAN R. PIRGOZLIEV BSc, MSc

A thesis submitted in partial fulfilment of the requirements of the Open University for the degree of Doctor of Philosophy

October 2002

Harper Adams University College in collaboration with BASF Plc UK

To my son Radislav, with all my love

Acknowledgements

I would like to thank my Director of Studies Dr Pete Jenkinson and my Supervisors Dr Simon Edwards and Dr Martin Hare for their help and encouragement. I would like to thank BASF Plc UK as my sponsoring organisation and in particular Dr Jo Sykes. I would like also to thank Dr Phil Jennings from Central Science Laboratory, York, for supplying the project with *Fusarium* isolates and Professor Naresh Magan from Cranfield University for the saprophytic isolates. I would like to thank the whole of the CERC gang for helping me to carry out field and glasshouse experiments, and in particular Jan, Allison, Dave, Kate and Charles. I would like to thank the Library and Computer teams for helping me to obtain valuable information on my subject and also John Pick for the nice pictures.

In addition, I would like to thank my student colleagues at Harper Adams; in particular Ruth, Allison, Sarah, Rob, Claire, Shoko, Rumiana, Ian, James, Mat and Jacqueline for their help and friendship. I also would like to thank my Mum, Dad and my brother Vasil who were always there for me.

Abstract

In order to determine the effect of a range of fungicide products, dose rate and time of application on the development of Fusarium head blight (FHB) and mycotoxin contamination of winter wheat, a series of field experiments (1998-2001) were carried out. A mixture of the fungicides metconazole+azoxystrobin provided the most significant reduction of (FHB) severity (63%) in comparison with control treatments followed by mectonazole (39%) and tebuconazole (45%) applied at both full and half the manufacturers recommended dose rate. Quantification of *Tri*5 DNA and deoxynivalenol (DON) concentration in harvested grain indicated that metconazole and tebuconazole were the most efficacious fungicides at reducing colonisation of grain by trichothecene-producing *Fusarium* species and DON content. Azoxystrobin had no effect on *Tri*5 DNA and DON concentration in grain, but significantly reduced the incidence of *Microdochium nivale* infected wheat grain. Fungicides applied early in the growing season (GS 31 and 39) were less effective at controlling disease than those applied later in the season (GS 39 and 59). Indeed, it was evident that the timing of fungicides was critical for the effective control of FHB and DON with the best control achieved when fungicides were applied either two days before or two days after the artificial inoculation of wheat ears with FHB causing pathogens.

Regression analysis on field trial data revealed that fungicides were able to influence DON content in harvested wheat grain by affecting the extent of grain colonisation by mycotoxin-producing species. Since published literature has suggested that fungicides can directly influence DON contamination by imparting a stress influence on FHB pathogens, glasshouse studies were undertaken to determine the effect of a range of dose rates of the fungicides azoxystrobin and metconazole against FHB and DON in wheat plants inoculated with either *Fusarium culmorum* or *F. graminearum*. Results revealed that metconazole was effective at reducing FHB, *Tri*5 DNA and DON and that a rate response was observed as dose rate was increased from one quarter to double the manufacturer's recommended dose rate. Although azoxystrobin reduced FHB, *Tri*5 DNA and DON content compared to untreated controls, no dose rate response was observed. Regression analysis of glasshouse data supported those obtained from field studies, suggesting that fungicides

are able to influence DON content indirectly by affecting the extent of grain colonisation *by F. culmorum* and *F. graminearum.*

Further glasshouse studies were conducted to determine the interactions between *Alternaria tenuissima, Cladosporium herbarum, M. nivale* and *F. culmorum* and the effect of fungicides on these interactions. Introducing *C. herbarum* or *M. nivale* to wheat ears at GS 57, before inoculation with *F. culmorum* at GS 65, resulted in a significant increase in FHB severity, *Tri5* DNA and DON concentration in grain. Applying azoxystrobin to wheat ears after the introduction of *M. nivale* at GS 57, increased DON concentration in grain by 56%. The significance of interactions between saprophytic fungi, *M. nivale* and *Fusarium* species on fungicides efficacy against FHB and DON is discussed.

LIST OF PUBLICATIONS

Papers

Pirgozliev SR, Edwards SG, Hare MC and Jenkinson P, 2002. Effect of dose rate of azoxystrobin and metconazole on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain. *European Journal of Plant Pathology* **108**, 469-478.

Edwards SG, **Pirgozliev SR**, Hare MC and Jenkinson P, 2001. Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. *Applied and Environmental Microbiology* **67**, 1575-1580.

Conference presentations

Pirgozliev SR, Edwards SG, Hare MC, and Jenkinson P. 2001. Effect of range of fungicides and time of application on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in winter wheat. In: *Proceedings of the Second Workshop on Fusarium Head Blight*, Ottawa, Ontario, Canada, November 3-5, 2001, p.131.

Pirgozliev SR, Edwards SG, Hare MC and Jenkinson P. 2001. Effect of timing and fungicides on Fusarium head blight development and accumulation of deoxynivalenol (DON) in winter wheat. In: *Proceedings of: Sustainable Systems of Cereal Protection against Fungal Diseases as the Way of Reduction of Toxin Occurrence in Food Webs*, Kroměříž, Check Republic, July 2-6, 2001, p. 221-224.

Pirgozliev SR, Jenkinson P, Edwards SG, and Hare MC. 2000. Effect of metconazole and azoxystrobin on the development of Fusarium ear blight and the accumulation of deoxynivalenol (DON) in winter wheat. In: *Proceedings of 6th European Fusarium Seminar*, Berlin, Germany, September 11-16, 2000, p. 103.

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Chapter 1

Introduction and literature review

1.1 Introduction

Fusarium Head Blight (FHB), also known as ear blight or scab, is a significant disease of small grain cereals and has been reported throughout the world. The disease affects yield in cereals through reduction of thousand-grain weight, specific grain weight and grain number per ear. In addition, due to the ability of several FHB pathogens to produce mycotoxins, the disease poses serious threats to the health of humans and animals.

1.2 Incidence of FHB

Early reports in the USA by Arthur (1891) showed that scab infection of wheat fields in Indiana ranged from 25-75%. According to Atanasoff (1920), during 1917, scab was found in 31 of 40 states in the USA and during 1919, a severe scab outbreak caused grain losses throughout the United States amounting to approximately 80,000,000 bushels. Incidence of the disease varied from state to state from just trace levels in Alabama and Arkansas to 34% in Illinois and 69% in Iowa (Dickson and Mains, 1929). McMullen and Nelson, (1995) during a survey of 161 wheat fields in the state of North Dakota in 1994 observed that in the north east district of the state, 60% of crops were affected by scab with up to 76% ears infected in some fields.

In Canada, FHB has been observed throughout most of the wheat growing areas but the most severe outbreaks have been reported in Ontario, The Maritime Provinces, Manitoba and Peace River Region of Alberta (Sutton, 1982). Tekauz *et al.* (1988) assessed 122 wheat fields in south-central Manitoba and found 55% incidence of FHB. Out of these, 46% of fields of common wheat, 72% of durum and 61% of semi-dwarf wheat were affected by FHB. Wong *et al.* (1992) during a 4-year survey between 1989 and 1991 involving 436 wheat fields in Manitoba found symptoms of FHB in 308 fields (70%). Severity of disease, assessed as the percentage of kernels infected, varied significantly between years. During 1991, when FHB outbreaks were severe, 18% of hard red spring wheat crops, 37% of semi-dwarf wheat crops and 25% of durum wheat crops were affected.

In Hungary, a survey based on the investigation of seed infection of wheat samples taken from up to 700 sampling sites from all over the country showed that seed samples in 9 years out of 27 (1970-1996) had heavy infection between 15-25% indicating high incidence of FHB (Apony *et al.*,

1998). Although in the other years, seed infection was between 4.6% and 13.5%, it was noted that in certain wheat growing districts up to 40% of grain was infected with *Fusarium* spp. In the Netherlands, national surveys involving between 100-170 winter wheat crops from 1974 to 1986 showed that an average of 51% of fields were infected with FHB with an average of 1.2% glumes infected (Daamen *et al.*, 1991). A national survey of 356 wheat crops in the UK undertaken by Turner *et al.* (1999) in 1998 showed that more than 60% of the crops were affected by FHB with an average 12% of ears infected.

Incidence of FHB has also been reported in Asia. For example a 12-year (1951-1985) survey in China in wheat growing regions of the Yangtze river valley showed between 50-100% FHB incidence in severe epidemic years and between 20 and 40% in moderate epidemics (Zhuping, 1994).

1.3 Causal organisms and Geographical Distribution

FHB has been identified world-wide and up to 17 species have been associated with the disease including *Fusarium acuminatum, F. anthophilum. F. avenaceum, F. culmorum, F. dimerum, F. equizeti, F. graminearum, F. merismoides, F. oxysporum, F. poae, F. sacchari, F. sambucinum, F. solani, F. sporotrichioides, F. tricinctum, F. verticillioides* and *Microdochium nivale* (formerly *F. nivale*) (Parry *et al.*, 1995a). However, only five are considered as significant FHB causal organisms: *F. culmorum, F. graminearum, F. avenaceum, F. poae* and *Microdochium nivale.*

A survey of wheat grain in England, Scotland and Wales in 1989 by Polley *et al.* (1991) showed about 5% infection with *F. culmorum*, 4% with *F. poae*, 3% with *M. nivale* and 1% with *F. avenaceum.* In the following year in Scotland, 2.5% of grain was infected with *F. poae*, 4% with *M. nivale*, 1% with *F. avenaceum* and less than 1% with *F. culmorum.*

More recently, Jennings *et al.* (2000) took grain samples from 53 severely infected wheat fields: 6 located in Wales and 47 sites in England. The predominant head blight pathogen was *M. nivale* var.

majus present in 94% of the samples. The remaining head blight pathogens, *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* were present in 43%, 21%, 40% and 34% respectively. In Ireland over a period of 25 years (1930-1954) the most common species causing seedling blight, brown foot rot and head blight were *F. culmorum* and *M. nivale* (McKay, 1957).

Fusarium species vary with geographic location in accordance with their environmental preferences. For example, since the beginning of the 20th century in the continental climate of the USA all serious outbreaks of scab reported in small grain cereals were due to *Fusarium graminearum* as a predominant causal organism (Dickson and Mains, 1929; Dickson, 1942; Boosalis *et al.*, 1983; Salas *et al.*, 1999). *Fusarium graminearum* also has been recorded as the main FHB pathogen in countries with a climate similar to the USA such as Canada (Wong *et al.*, 1992), Australia (Burgess *et al.*, 1987), Argentina (Moschini *et al.*, 2002), Russia (Gagkaeva and Levitin, 1997) and China (Zuping, 1994).

According to Bottalico and Logrieco (2001), in Europe, the predominant species responsible for FHB are *F. graminearum*, *F. culmorum* and *F. avenaceum*. However, the frequency of these species may vary with the geographical location of the country. Mesterhazy (1984) during a 13 year survey (1970-1983) identified *F. graminearum* and *F. culmorum* as the predominant species from diseased wheat samples in South Hungary. Surveys in Austria (Adler *et al.*, 1990) and Bavaria (Rintelen, 1992) showed that *F. poae* and *F. avenaceum* tended to predominate followed by *F. graminearum*. However, later reports by Obst *et al.* (2000) showed that *F. graminearum* has prevailed in wheat in Bavaria. Daamen *et al.* (1991) in Holland during a nine year survey considered *F. culmorum* and *Microdochium nivale* to be the predominant species isolated from blighted winter wheat ears. Balmas *et al.* (1999) and Pasquini *et al.* (2001) both frequently isolated *F. graminearum*, *F. avenaceum* and *F. culmorum* from wheat heads collected from central and northern regions of Italy. In France during 1996, *F. culmorum* was reported by Bakan *et al.* (1998) as the predominant species in wheat. In 1998, Chełkowski *et al.* (2000) reported *Fusarium avenaceum* to be the predominant species in cereals throughout Poland. *Fusarium avenaceum* seems to be more tolerant to various temperature and moisture conditions, since it has been

frequently isolated from wheat heads in India (Chaudhary *et al.*, 1990) from Norwegian cereals (Henriksen *et al.*, 2000), and grain samples from Alberta in 1999-2001 (Turkington, 2001).

There is evidence for the existence of distinct sub-populations in *F. graminearum* and *M. nivale*. With regards to the ability of *F. graminearum* isolates to form or not to form perithecia, Francis and Burgess (1977) in Australia characterised the populations of the species into two groups. Isolates, which did not produce perithecia *in vitro* and very rarely in nature, belonged to Group 1. Those isolates that readily formed perithecia in culture and *in planta* belonged to Group 2. According to the authors, Group 1 isolates were mostly associated with crown diseases, and Group 2 isolates were related to diseases of aerial parts of the plants, including FHB. As a result of a recent morphological, physiological and molecular study, Aoki and O'Donnel (1999) reported that Group 1 strains, previously considered as a distinct population of *F. graminearum*, represents a distinct species, which the authors described as *Fusarium pseudograminearum*.

Studies of spore morphology of *M. nivale* by Gerlach and Nirenberg (1982) and by restriction fragment length polymorphism (RAPD) assay (Lees *et al.*, 1995) resulted in differentiation of two sub-groups of the species: *M. nivale* var. *majus* and *M. nivale* var. *nivale*.

1.4 The economic importance of FHB

FHB is a significant disease of small grain cereals in that not only does it reduce grain yield, but also due to the deterioration of grain derivatives and the production of mycotoxins by certain causal pathogens, can significantly reduce grain quality.

1.4.1 Effect on yield

There is a wealth of literature from all over the world regarding the effect of FHB on the yield in small grain cereals. For example, early reports made by MacInnes and Fogelman (1923) stated that severe scab epidemics during 1905, 1907 and 1915 in Minnesota accounted for grain losses in wheat crops of ca. 5%. In 1928, after prolonged wet weather during the blossoming of small grain cereals in Indiana, a scab epidemic caused yield reductions of 15% in wheat, 20% in barley and 3%

in oats (Mains *et al.*, 1929). Another extensive field survey of wheat crops in the Atlantic Provinces of Canada during 1980 by Martin and Johnston (1982) revealed that FHB was responsible for between 30% and 70% yield loss. Scab epidemics in wheat and barley occurred in southern Idaho in 1982 and 1984 and resulted in estimated yield losses as high as 50% in some fields (Michuta-Grimm and Foster, 1989). According to Sayler (1998) in nine US states between 1991 and 1996, wheat producers lost 501 million bushels of grain, equivalent to $ 2.6 billion. Hard red spring wheat crops were worst affected with ca. 52% production losses, whilst soft red wheat and durum wheat experienced 38% and 10% production losses, respectively.

According to McKay (1957), a severe head blight outbreak in Ireland in 1942, decreased yield in wheat by between 21 and 55% and a second outbreak during 1954 was responsible for yield reductions in wheat and oat crops by up to 50%. In Romania, Munteanu *et al.* (1972) and Tusa *et al.* (1981) reported that in epidemic years, FHB of wheat caused losses of approximately 40 % in some regions of the country, with up to 70% losses in some fields. As a result of natural infection of wheat by *F. avenaceum* in India, Chaudhary *et al.* (1990) reported yield losses between 15 and 29%. In China, the largest area affected by FHB is in the mid and lower regions of the Yangtze river valley. Surveys carried out in this area between 1951 and 1985 recorded 19 FHB outbreaks with grain yields of wheat reduced by 5-15% in years when moderate epidemics of FHB were recorded and up to 40% in years when disease epidemics were severe (Zhuping, 1994).

Although these surveys provide an indication of the potential yield loss that may be associated with FHB, they provide no indication as to how the disease reduces grain yield. More precise data on the effect of FHB on grain yield has been obtained from artificially inoculated field trial studies. Duben and Fehrmann (1979), for example, whilst investigating the pathogenicity of *Fusarium* spp. on winter wheat in Germany, found that after artificially inoculating plots of winter wheat with *F. culmorum*, *F. graminearum* and *F. avenaceum*, grain yield was significantly reduced and that such losses were strongly related to a reduction in individual grain weight. Arseniuk *et al.* (1993) reported that under experimental conditions, 1000-grain weight, the number of grains per head and head weight in four triticale varieties were reduced by 15, 18 and 22% respectively. Following the

artificial inoculation of double haploid barley genotypes with *Fusarium culmorum*, Surma *et al.* (2000) observed significant reductions in kernel number per ear (4-31%) and 1000 kernel weight (14-31%) when compared to uninoculated plots of the same barley genotypes.

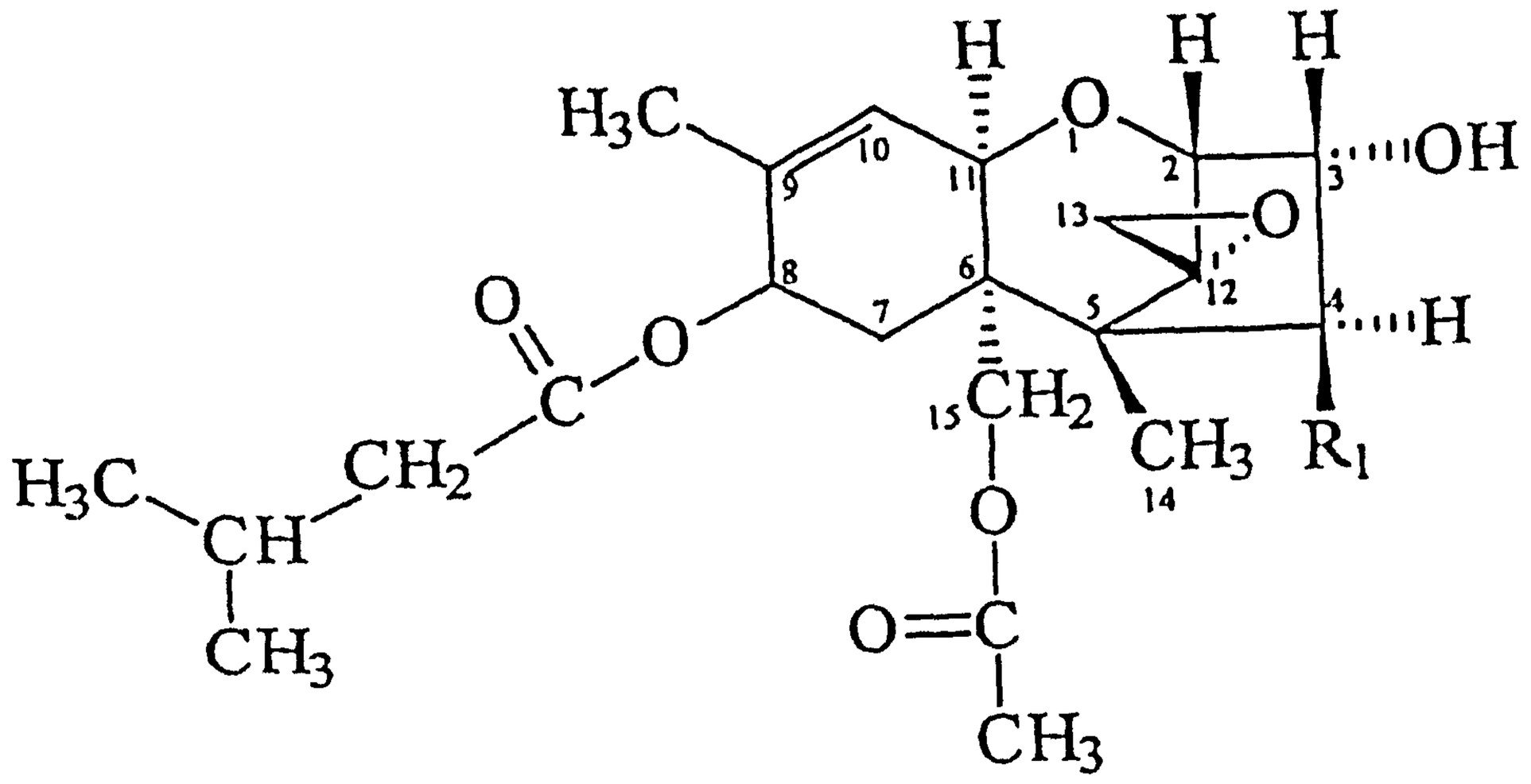
1.4.2 Effect on grain quality

The presence of *Fusarium* spp. in wheat can cause deleterious effects on grain processing qualities. Bechtel *et al.* (1985) for example, found that *F. graminearum* was capable of destroying starch granules, storage proteins and cell walls during wheat kernel invasion. Dexter *et al.* (1996) showed that Canadian hard red spring wheat grain samples, which contained *Fusarium* damaged kernels, exhibited weak dough properties and unsatisfactory baking quality. Studying the effect of fungal proteases on wheat storage proteins, Nightingale *et al.* (1999) suggested that *F. graminearum* and *F. avenaceum* produce proteolytic enzymes capable of digesting wheat storage proteins. These enzymes hydrolyse endosperm proteins during dough mixing and fermentation and result in weaker dough and decreased loaf volume.

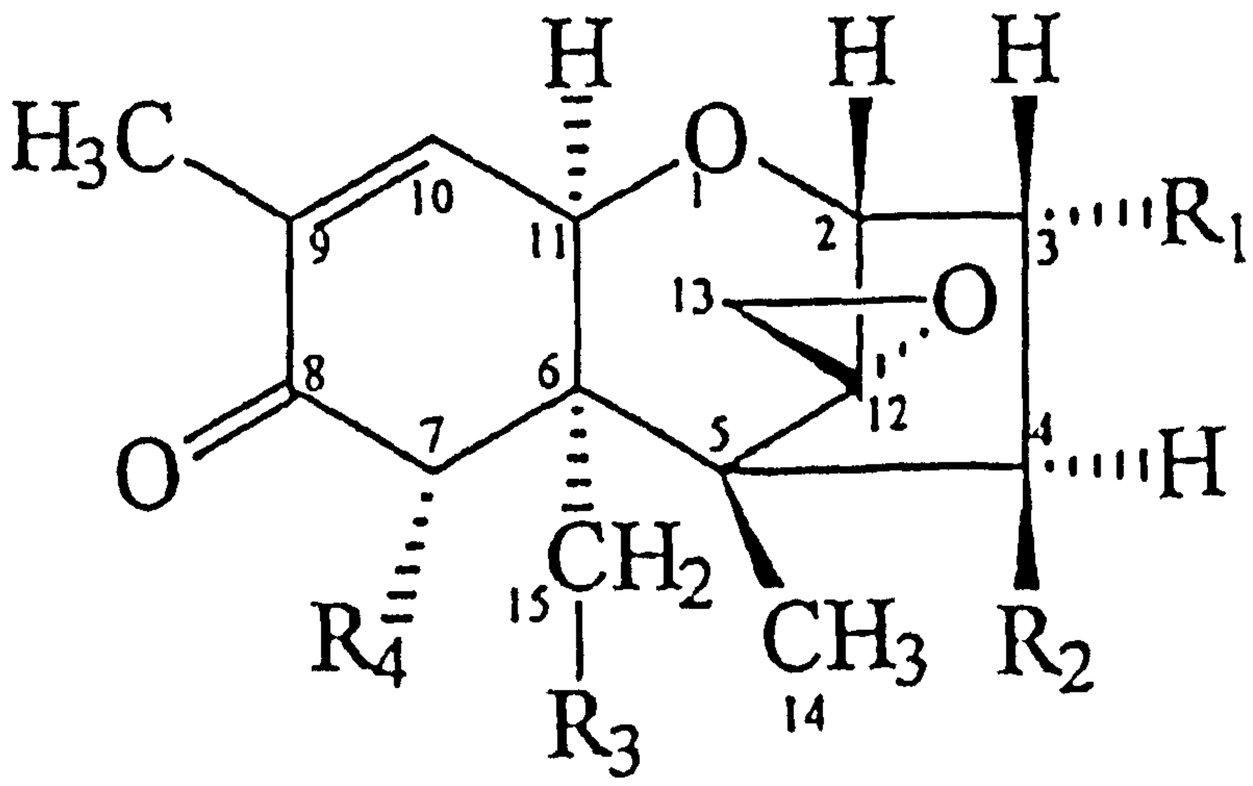
In barley, grain infected with *Fusarium* spp. has been demonstrated to cause a reduction in germination and subsequent malt yield as well as causing uncontrolled foaming of beer (gushing) during the malting process (Narziss *et al.*, 1990; Schwarz *et al.*, 2001). Odhav and Naicker (2002) whilst investigating mycotoxin content in 29 South African beers observed that 13 contained zearalenone at concentrations ranging from 2.6 to 0.426 $\mu g\ l^{-1}$.

Apart from effects on grain processing qualities *Fusarium* species are associated with the production of a range of toxic metabolites. These include a number of mycotoxins belonging to the trichothecene group of mycotoxins. The different trichothecenes produced by members of the *Fusarium* genus are classified as type A or type B according to the structural component at C-8 (Krska *et al.*, 2001). Type A trichothecenes include T-2 and HT-2 toxins whilst type B is represented by deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), and fusarenon-X (FUS-X) (Figure 1.1). Generally *F. sporotrichioides*, *F. poae* and *F. equiseti* produce type A trichothecenes, whereas *F. graminearum*,

F. culmorum and *F.crookwellense* are the most important species producing type B trichothecenes (Thrane, 1989). *Fusarium graminearum*, *F. culmorum* and *F. crookwellense* also produce an oestrogenic mycotoxin zearalenone (ZEN) also known as F-2 toxin (Figure 1.2) which does not

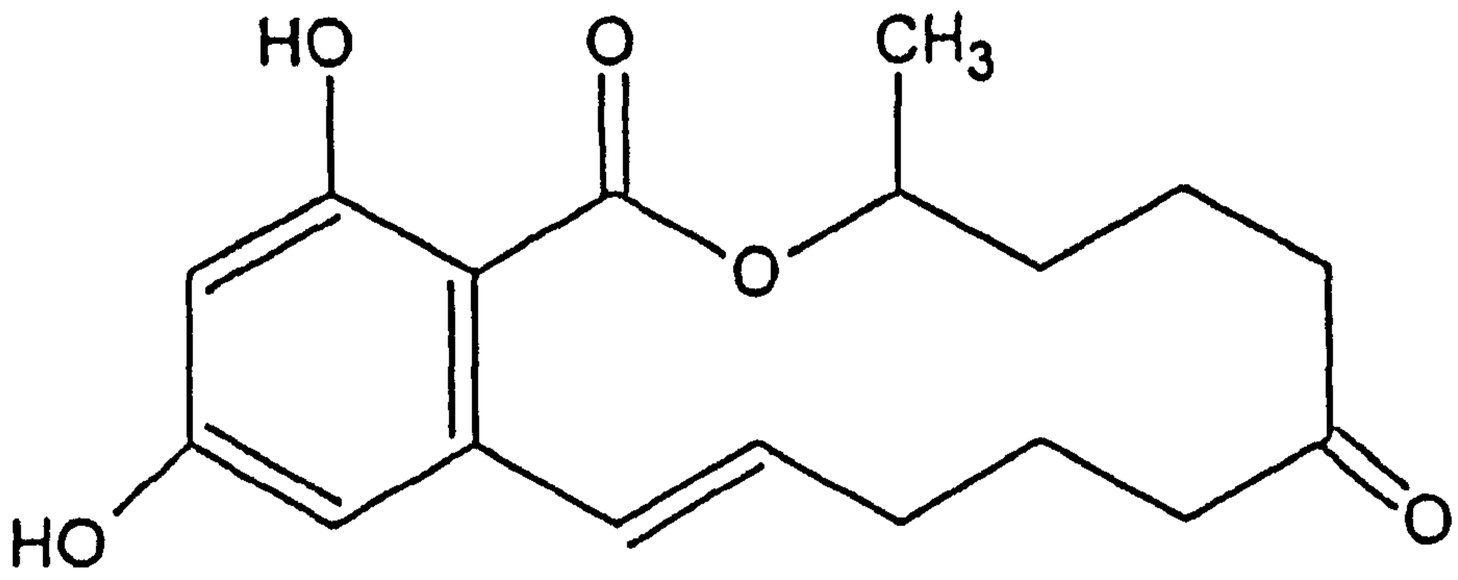


The structure of the type –A trichothecenes: T-2 toxin (R_1=OAc) and HT-2 toxin (R_1=OH)



The structures of type –B trichothecenes: DON (R_1=OH, R_2=H, R_3=OH, R_4=OH), NIV (R_1=OH, R_2=H, R_3=OH, R_4=OH), 3-AcDON (R_1=OAc, R_2=H, R_3=OH, R_4=OH), 15-AcDON (R_1=OH, R_2=H, R_3=OAc, R_4=OH) and FUS-X (R_1=OH, R_2=OAc, R_3=OH, R_4=OH)

Figure 1.1 The structures of type-A and type-B trichothecenes (From Krska *et al.*, 2001)



Zearalenone (ZEN)

Figure 1.2 Chemical structure of zearalenone (From Hussein and Brasel, 2001).

belong to the trichothecene group of toxins (Hussein and Brasel, 2001). *Fusarium avenaceum* other toxins such as moniliformin, Fusarin C and eniatins (Bryden *et al.*, 2001).

If grain contaminated with *Fusarium* toxins is used as feed or for human consumption, this may cause a range of adverse toxicoses as well as other health disorders. For example, in the middle of the last century in Russia, the consumption of food prepared from over-wintered cereals, contaminated with *F. poae* and *F. sporotrichioides*, caused human poisoning known as Alimentary Toxic Aleukia (ATA) where symptoms of fever, necrotic angina, leukopenia, haemorrhaging, exhaustion of bone narrow and in some cases death were observed (Joffe, 1978). In 1987, an outbreak of gastrointestinal disorder which occurred in the Kashmir Valley, India was associated with the ingestion of *Fusarium* mycotoxins (Bhat *et al.*, 1989). Of 150 families which were interviewed, 39 were shown to have had family members affected by the disease. Subsequent epidemiological investigations showed that all samples of wheat grain and flour tested during 1987 contained DON at concentrations ranging from 0.43 to 8.38 mg kg^{-1}. Similarly, in China, 53 outbreaks of human food poisoning associated with scabby and mouldy cereals occurred between 1960 and 1991 (Luo, 1992). In the Anhui province in 1991, approximately 130,000 people were affected by gastrointestinal disorders, accompanied with abdominal pain, nausea, vomiting, fatigue and fever (Huang, 1992). Analysis of eight wheat and two barley samples taken from affected

households in the Anhui province by Li *et al.* (1999) revealed that DON was present in all samples at concentrations ranging from 0.016 to 51.45 mg kg^{-1}. Nivalenol was also detected in all eight wheat samples and one of the barley samples (0.001-6.93 mg kg^{-1}). Furthermore, both barley samples and six wheat samples contained zearalenone at concentrations of between 0.046 and 0.3 mg kg^{-1}. More recently, in 1998 and 1999, Li *et al.* (2002) analysed wheat samples taken from crops in the Henan Province of China, where previously, cases of human toxicoses had been reported (Luo *et al.*, 1987). Thirty out of the 31 samples tested (97%) from Puyang area of these province, contained DON with a mean concentration of 2.8 mg kg^{-1}, however, some samples had up to 14 mg kg^{-1} DON concentration. Twenty one of the samples tested (70%) were shown to exceed the Chinese regulations of 1 mg DON kg^{-1} grain.

In animals the effects of DON vary depending upon the species involved, severity and duration of exposure to contaminated grain (Rotter *et al.*, 1996). Among the farm animals, swine show greatest sensitivity to DON, while poultry and ruminants, appear to show higher tolerance to the toxin (Trenholm *et al.*, 1984). In feeding trials where swine, poultry and dairy cattle were fed DON-contaminated wheat, these authors found that swine could ingest up to 2 mg of DON kg^{-1} grain without serious adverse effects. Poultry could tolerate up to 5 mg DON kg^{-1} feed while dairy cattle could tolerate up to 6 mg of DON kg^{-1} feed. Studies on vomiting in pigs when fed wheat grain naturally infected with *F. graminearum* and containing on average of 23 mg kg^{-1} DON and 4 mg kg^{-1} zearalenone, carried out by Williams *et al.* (1988) showed that vomiting commenced between 10 and 60 minutes after feed consumption. This was accompanied by signs of abdominal distress, teeth grinding and a marked feed refusal. Rotter *et al.* (1995) later demonstrated that such feed refusal in pigs associated with DON-contaminated grain accounted for a 13% lower weight gain when compared to the performance of pigs fed uncontaminated grain. Blood samples taken form swine on the same diet showed transient changes in serum proteins.

Hamilton *et al.* (1985a, 1985b, 1986) carried out several trials with poultry using dietary levels of DON up to 8 mg kg^{-1}. These workers found that DON-contaminated grain had no effect on either feed intake, body weight gain or any other of a number of productivity parameters recorded. The

studies of both Trenholm *et al.* (1985) who fed grain contaminated with 6.4 mg kg^{-1} DON and Côte *et al.* (1986) who fed grain contaminated with 6.6 mg kg^{-1} DON to dairy cows revealed no signs of either illness or reduced performance. Such observations may be in part explained by the ability of rumen inhabiting micro-organisms to metabolise or detoxify the toxin compounds (King *et al.*, 1984).

The oestrogenic compound zearalenone has been shown to cause a range of reproductive disorders in young pigs ranging from vulva vaginitis and vaginal prolapses to enlargement of the uterus and atrophy of the ovaries (Mirocha *et al.*, 1971). Studies of Miller *et al.* (1973) showed that consumption of grain contaminated with zearalenone by pregnant sows resulted in an increase in stillborn pigs and small litters. T-2 toxin has been shown to cause reductions in feed consumption and weight gain due to severe oral lesions in chickens (Kubena *et al.*, 1994). The toxin has also been associated with coagulopathy (Doerr *et al.*, 1981) and altered feathering (Wyatt *et al.*, 1975).

As a result of these adverse side effects induced by trichothecene mycotoxins, many countries have put into place proposals for legislative measures to ensure minimum levels of contamination of grain by these toxins. According to van Egmond (1989), Canada, USA, Rumania and Russia are countries which all have guidelines for DON concentration in finished products for human consumption and for animal feeds. Within the European Union, proposals for advisory limits set for DON concentrations found in cereal grain are being discussed. Such advisory limits are 500µg DON kg^{-1} for retail products such as breakfast cereals, bread and pasta and 750µg kg^{-1} for raw grain and flour (Pricket *et al.*, 2000).

Although trichothecene mycotoxins are primarily associated with field contamination and development, inappropriate conditions employed during the storage of grain can result in further increases in mycotoxin content. For example, Birzele *et al.* (2000) determined the influence of delayed drying of wheat grain after harvest on the development of DON and ochratoxin A during grain storage trials. Wheat grain was stored at 20°C at two regimes of 17% and 20% grain moisture content for a period of six weeks. Although the initial DON concentrations in grain for the two years

studied was high, ranging from 0.04 to 0.41 mg kg^{-1} in 1997 and between 0.67-1.02 mg kg^{-1}, these workers observed a significant increase on DON concentration up to 2.186 mg kg^{-1} in 1997, and up to 2.986 mg kg^{-1} in 1998 when grain was stored at 20% moisture content. Storing grain at 17% grain moisture also led to a considerable increase in DON contamination. Interestingly, despite such increases in DON contamination, quantification of *Fusarium spp.* in grain over the storage period revealed a significant decrease in the colonisation of grain by these pathogens when stored at either 20 % or 17% moisture content.

Work by Homdork *et al.* (2000a) also confirmed that inappropriate conditions employed during the storage of grain could result in further increases in mycotoxin content. In storage trials, when grain with different initial levels of infection by *F. culmorum* (4%, 15%, 15% treated with tebuconazole and 52%) was kept under different storage conditions (15°C/56% RH (Relative Humidity), 25 °C/73 % RH, 25 °C/90% RH; 15°C/56% RH, 15 °C/73% RH, 15 °C/84% RH, 25 °C/62% RH, 25 °C/73% RH and 25 °C/90% RH) for 36 weeks. In the end of the storage period DON concentration in 4% and 15% infected grain under 25°C/90% RH increased up to 19.33 and 3.27 mg kg^{-1} respectively. Unlike DON concentrations in grain nivalenol and zearalenone had increased up to 2.25 and 5.07 mg kg^{-1}, respectively, under 25 °C/90 % RH storage condition and 52% infection level. Cool and dry conditions (15°C/56% RH) maintained good seed quality but the level of grain infection remained unchanged.

Fusarium toxins have been reported in many countries in the world. For example Scott *et al.* (1981) recorded DON concentrations of between 0.01 and 4.3 mg kg^{-1} in 44 of 45 samples of winter wheat from Ontario, and between 0.01-0.03 mg kg^{-1} in all 27 red spring wheat samples tested. Between 1991 and 1998, Campbell *et al.* (2000) collected 166 barley samples from throughout eastern Canada and found that 84 of them were contaminated with DON. In some of the samples, concentrations of up to 9 mg of DON kg^{-1} grain were recorded. Blaney *et al.* (1987), between 1983 and 1985, analysed samples taken from nine grain stores and grain depots in south-eastern Queensland, Australia. DON was detected in nearly all pooled samples ranging from trace levels of less than 0.01 up to 1.7 mg kg^{-1} grain. In a few samples, DON concentrations of up to 11.7 mg kg^{-1}

grain were recorded. During a survey of mycotoxins present in wheat and maize from western Romania, Cutui *et al.* (1998) detected DON in all 25 wheat and 46% of 30 maize samples tested. DON concentration in wheat grain varied from 0.097 to 0.56 mg kg^{-1}. In a review of world-wide contamination of cereals and animal feedstuff with *Fusarium* mycotoxins, Placinta *et al.* (1999), reported DON concentrations in wheat grain up to 40 mg kg^{-1} in samples from Germany, Poland, Japan, New Zealand, USA, and Argentina. Samples of barley grain from Norway, Japan and USA were found with DON concentrations of up to 71mg kg^{-1}. Clear *et al.* (2000) during 1996 and 1997, collected 1494 barley samples from Western Canada. Concentrations of DON higher than 0.05 mg kg^{-1} were found in barley samples from 7 out of 10 Manitoba districts and 1 out of 19 Saskatchewan districts in 1996, and from 8 of 11 Manitoba and 1 of 20 Saskatchewan crop districts in 1997.

Using High-Performance Liquid Chromatography (HPLC) Fazekas *et al.* (2000) quantified DON concentration in 99 feed wheat samples grown in north-eastern Hungary. DON was detected in 88% of the samples tested. On average, DON concentration was 0.94 mg kg^{-1} grain, however, 4.3 mg DON kg^{-1} grain was detected in one sample.

1.4.3 Effect on seed quality

The drilling of *Fusarium* infected cereal seed has been linked with the subsequent development of seedling blight and foot rot in growing crops (Nelson, 1929). According to Betchel *et al.* (1985) when *F. graminearum* invades wheat grain, significant destruction of the starch granules, storage proteins and cell walls occurs resulting in reduced germination and vigour. Sowing seed taken from a field trial artificially inoculated with *F. graminearum*, Wong *et al.* (1992) reported that seed germination and subsequent plant establishment was reduced by up to 50%. Studies in Ireland (Humphreys *et al.*, 1995) showed that levels up to 79% of seed-borne infection with *Microdochium nivale* wheat seed resulted in substantial reduction in plant establishment (62%), number of ears per m^{-2} (32%) and grain yield (40%). More recently, during a field trial involving six winter sown oat varieties, Humphreys *et al.* (1998) observed a strong correlation between the amount of seed-borne *M. nivale* in seed samples drilled and plant establishment ($r^2 = 0.83$).

1.5 Symptoms of FHB

Atanasoff (1920) provided the first detailed description of FHB symptoms caused by *Gibberella saubinetii* (*Gibberella zeae* teleomorph form of *F. graminearum).* According to this author, initial symptoms are seen as a slightly brown water-soaked spot in the length of the glumes. Over time the lesion size increases until the whole spikelet is covered, and, depending on weather conditions spreads to the neighbouring spikelets. Infected plant tissue then begins to senesce, taking on the typical colour of a ripe head in contrast with green healthy uninfected heads. In some cases, infection on the rachis causes blighting or death to those spikelets situated above the point of infection. The production of sporodochia at the base of infected glumes gives rise to a pinkish colour on severely infected ears. Characteristic symptoms of FHB are shown in Plate 1.1.

Symptoms of FHB caused by *F. poae* and *M. nivale* are disputable. Polley *et al.* (1991) described the symptoms of *F. poae* as lesions with a bleached centre with a dark brown margin on the glumes. In contrast, Parry *et al.* (1995b) suggested that *F. avenaceum, F. culmorum* and *M. nivale* are also capable of producing *F. poae*-like symptoms during initial development of symptoms. Further work is, therefore, needed to determine the exact initial symptoms induced by each of the causal pathogens responsible for FHB. According to Rapilly *et al.* (1973), infection of ears by *M. nivale* causes brown spots with a dark brown margin to appear on glumes. Such observations contradict those of Cassini (1981) who suggested that FHB caused by *M. nivale* exhibit symptoms that cannot be distinguished from those caused by *F. roseum* (*F. culmorum, F. graminearum* and *F. avenaceum*).

Grain harvested from FHB affected ears are often shrivelled and may have a red discoloration due to the presence of fungal growth. In North America, *Fusarium* damaged kernels (FDK) are assessed and categorised according to their colour; white tombstone (shrivelled, white and chalky) and pink tombstone (shrivelled, with pinkish appearance) (Sinha and Savard, 1997). Plate 1.2 shows typical symptoms of *Fusarium* damaged kernels.

Plate 1.1 Symptoms of Fusarium head blight on winter wheat

Plate 1.2 *Fusarium* infected wheat grain (first row) compared to healthy wheat grain (second row).

1.6 Life cycle of *Fusarium* in small grain cereals

A generalised disease cycle of *Fusarium* spp in small grain cereals can be seen in Figure 1.5.

1.6.1 Sources of inoculum

A primary source of inoculum for the development of FHB is crop debris (Atanasoff, 1920; Sutton, 1982). In Minnesota, Windels and Kommedhal (1976) found 84% of corn stalks infected by *F. graminearum* in the autumn. In the following spring the infection was 61% followed by 31% infection after a complete year. Khonga and Sutton (1986) observed a similar pattern of plant residue infection under field conditions in Ontario. Almost a year after the harvest in June, winter wheat stem segments, spikelets and seeds at the soil surface had 16, 242 and 191 perithecia of *F. graminearum* respectively, in June, and 12, 427 and 611 perithecia respectively, in August. Gilbert (2001) showed that viability of *F. graminearum* in infected wheat seed was not affected when it was exposed on the soil surface or buried at 5 or 10 cm in the soil. Under field conditions, survival of *F. graminearum* ranged from 85 to 100% at all depths after 24 months.

The relationship between *Fusarium* infected crop residues from the preceding crop and FHB incidence the following year was clearly demonstrated by Koehler *et al.* (1924). For a period of three years (1919-1921) the authors surveyed fields in seven American states with various crop rotations including corn, wheat, rye, oats, clover and timothy. They found that when corn was followed by wheat there was 43.3% head infection. When wheat was sown after wheat, rye or oats, 29.3%, 27% and 22.7% incidence of FHB was observed, respectively. The percentage of head infection dropped dramatically when the preceding crop of wheat was clover or timothy, when the average head infection observed was 11.4% and 8.3%, respectively. FHB occurs as a part of a group of diseases of cereals including seedling blight, brown foot rot and head blight (Figure 1.). Seedling blight occurs mainly as a result of seed-borne inoculum although soil-borne inoculum can be also responsible for the disease. In Scotland, *M. nivale* has been reported as the main causal organism of seedling blight (Rennie *et al.*, 1983), whilst in the USA Warren and Kommedahl (1972) demonstrated that *F. graminearum* was the predominant species infecting wheat seedlings. In a specific survey of *Fusarium* species in stem base of winter wheat in the Midlands of the UK

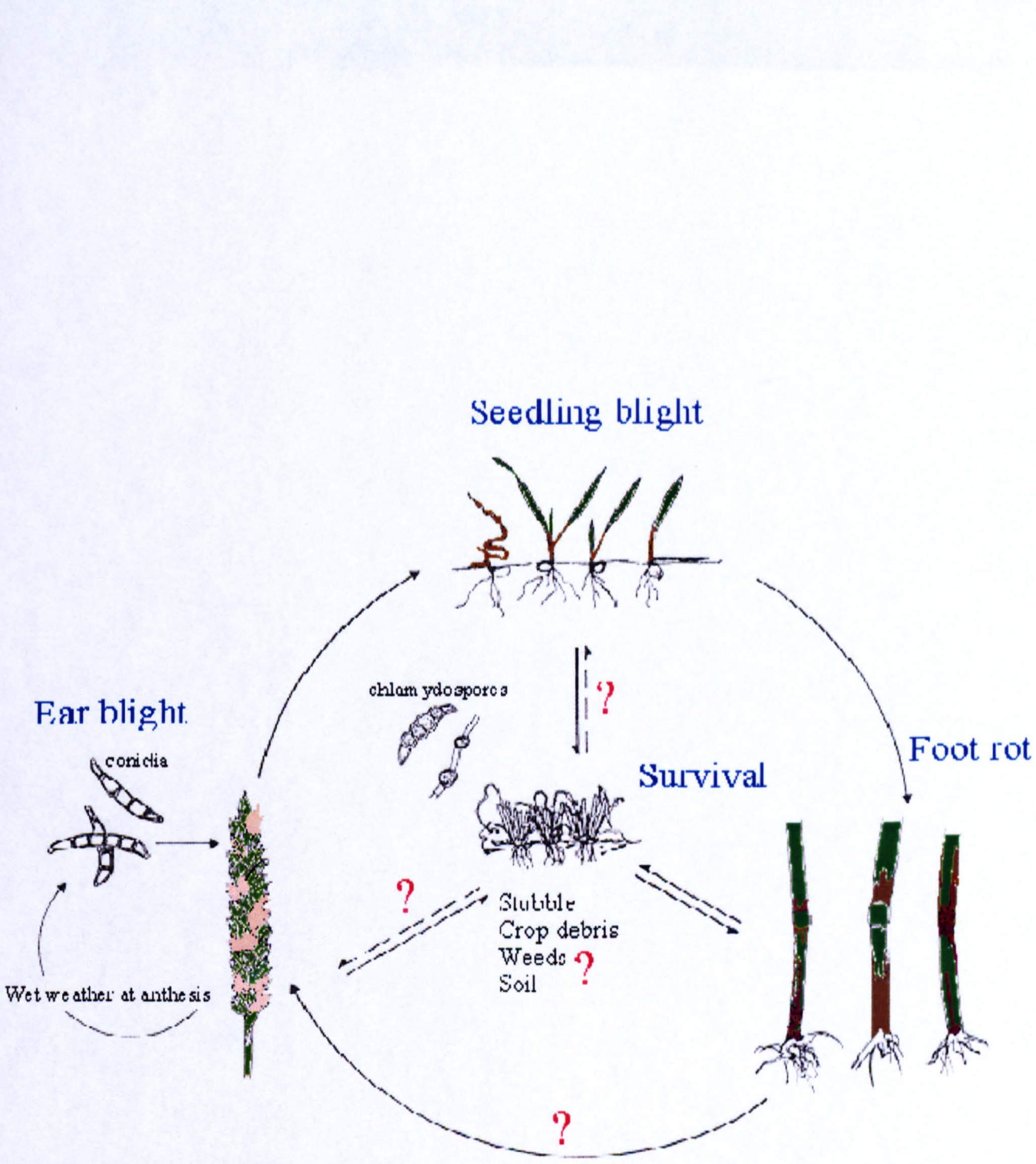


Figure 1.3 Generalised disease cycle of *Fusarium* on small grain cereals (From Parry *et al.*, 1995a).

between 1987 and 1989 wheat samples yielded up to 65% incidence of *M. nivale* and 60% *F. culmorum* (Parry, 1990). During a national survey on the incidence and severity of FHB in UK winter wheat crops between 1976 and 1988, more than 80% surveyed had foot rot (Polley and Thomas, 1991). The link between seedling blight, brown foot rot and FHB as a part of the Cereal Fusarium Complex (Figure 1.5) is not clear. However, the first two diseases may have the potential to provide the primary source of inoculum for head blight epidemics.

Grasses and broad-leaved weeds could be another important source of inoculum. McInnes and Fogelman (1923) reported that *Gibberella zeae* had occurred in Minnesota on western wheat grass (*Agropyron smithii*), quack grass (*Agropyron repens*) slender wheat grass (*Agropyron tenerum*) and wild rye grass (*Elymus spp.*). During a national survey in Canada, Gordon (1959) isolated *Fusarium* spp. from 19 species of cereals and grasses and 24 species of common weed plants. In the UK, Jenkinson and Parry (1994) isolated *Fusarium* spp. from 1346 broad-leaved weed samples representing 15 species from five genera (*Compositae*, *Rannunculaceae*, *Ploygonaceae*, *Cruciferae* and *Chenopodiaceae*). Despite no obvious symptoms of *Fusarium* infection, 226 isolates of *Fusarium* spp. were obtained of which 114 (50.4%) were *F. avenaceum*, 88 (38.9%) *F. culmorum*, 16 (7.1%) *F. poae*, 6 (2.5%) *F. sambucinum* and 3 (1.3%) were *F. graminearum*. Furthermore, of 77 isolates tested, 75 were shown to be pathogenic to winter wheat seedlings (cv. Mercia). Since these isolates were pathogenic to seedlings, it could be argued that alternative hosts such as weeds could provide a reservoir of inoculum for FHB epidemics and that weed control could provide beneficial effects in control strategies against FHB.

1.6.2 Dispersal of inoculum

Conflicting evidence exists regarding the mode of dispersal of *Fusarium* inoculum to the ears of cereals during FHB epidemics. For example, Jordan and Fielding (1988) indicated systemic infection of ears following artificial inoculation of wheat seedlings below soil level with *F. culmorum* and then re-isolating the pathogen from all internodes and some ears. Systemic growth of *F. culmorum* in the stem of winter wheat has been reported by Snijders (1990). After artificial inoculation of roots or seed of wheat with this pathogen, this author re-isolated the pathogen from

the stem tissue up to 70 cm above ground level. However, there was no evidence that systemic growth could cause ear infection. Work by Hutcheon and Jordan (1992) also suggested that systemic infection of wheat could lead to FHB. Placing mycelial plugs of either *F. avenaceum*, *F. culmorum*, *F. graminearum* or *M. nivale* at GS 21 of winter wheat, they re-isolated the same species from the basal node, internode 2 and internode 3 at GS 37 and GS 75 although plastic bags were placed on the wheat ears at GS 37.

Observations by other workers, however, give more convincing evidence that wind, rain and heavy dews appear to play a role in the dispersal of *Fusarium* infection to wheat ears. Atanasoff (1920) observed that the incidence of FHB in rye was more common in exposed areas in the direction of the prevailing wind, whereas FHB was less common in areas sheltered by wind breaks. In an investigation of the amount of rainfall and spore dissemination of *Gibberella zeae*, Hsia *et al.* (1958) found that this fungus produce more ascospores than conidia and the largest number of spores were trapped at 17 cm above ground and far fewer at 33 or 117 cm. The authors considered splashing rain to be more important for dissemination than wind. In the UK, Millar and Colhoun (1969) used "Rotorod" sampler to investigate the spread *M. nivale* in wheat. During dry weather periods, only a few spores were caught. After rain, the concentration of spores increased between 10 and 20 times with a peak 10 minutes after rain had stopped. Ascospores were trapped at 15 cm above ground. However, no spores were trapped as high as ear height. Diurnal release of ascospores of *Gibberella zeae* in wheat has been observed by Paulitz (1996). In plots inoculated with *G. zeae* colonised corn ascospore release began around 1600 to 1800 hours, reached a peak before midnight and declined to low levels by 0900 hours the following morning. Ascospore release occurred before leaf wetness and the peak release occurred 2 to 4 hours after heavy rainfall.

There is evidence that spores of *Fusarium* species can be splashed vertically and be dispersed to the wheat ear. Jenkinson and Parry (1994) showed that large incident rain drops (4-5 mm) could propel conidia of *F. culmorum* and *F. avenaceum* as high as 60 cm and 45 cm vertically and at maximum horizontal distances of greater than 100 and 90 cm, respectively. *Fusarium* inoculum may be carried towards the ear in a series of 'leaps' involving upper plant parts. Zinkernagel *et al.*

(1997) investigated the spread of *Fusarium* spp. from just above ground level to wheat ears. During three years, samples of internodes, leaves, leaf sheaths and ears were examined. The data showed that the disease incidence was higher on the leaf sheaths in comparison with stem bases and the predominantly isolated species was *F. graminearum*. Conidia were found at first on the lower leaves but as the season progressed, conidia has been detected on the upper leaves and ears. It was not clear from this work which was the main source of inoculum for FHB infection, infected leaves or air-borne *F. graminearum* spores. However, leaf infection should not be underestimated as a disease source.

Arthropod vectors seem to be another way of transmitting *Fusarium* spp. in field. Windels *et al.* (1976) recovered *Fusarium* spp. from adult picnic beetles (*Glischrochilus guardrisignatus* (Say)) collected from maize ears in Minnesota. *Fusarium* species have also been recovered from buried maize ears and from larvae, pupae and adults of picnic beetles. In Canada, a correlation between the occurrence of *F. graminearum* and wheat midges (*Sitodiplosis mosellana*) on wheat grain has been observed (Couture *et al.*, 1995; Morgain *et al.*, 1997).

1.7 Epidemiology of FHB

Growth stage of the host, wet weather and high temperatures appear to be critical for the successful infection of wheat ears by *Fusarium* species. For example, Jenkinson (1994) demonstrated that as wheat plants matured, they become less susceptible to infection by a number of *Fusarium* species. During controlled environment studies, wheat plants inoculated with either *F. avenaceum*, *F. culmorum* or *F. poae* at GS 60 (early anthesis) showed 37%, 53%, 17.3% spikelets infected, respectively. Plants inoculated at GS 65 and GS 70 with *F. culmorum* showed 33 and 13% spikelets infected whilst *F. avenaceum* and *F. poae* caused 13% and 4.9% and 5.6 and 3.1% spikelets infected for both growth stages, respectively. Recent work by Lacey *et al.* (1999) provided convincing evidence that host maturity and duration of wetness period play an important role in FHB development. After inoculating field plots with *F. culmorum* at GS 59, 65, 69, 71, 73, 75 and 77 and mist irrigating them for 0, 0.5, 1, 2 or 3 days, these workers observed that inoculation between GS 65-69 resulted in most severe symptoms of FHB when compared to earlier or later

inoculations. Plots inoculated at GS 65 and 69 and mist-irrigated for 0, 0.5, 1 and 3 days showed an increase in the incidence of infected ears from 2, 3.5, 6 and 16% and from 3, 5, 12 and 17.8% respectively. The incidence of FHB symptoms decreased significantly when plots were inoculated at GS 59, 73, 75 and 77.

The increased susceptibility of wheat plants to head blight infection during flowering suggests that anthers may play an important role in the initial infection of FHB pathogens. Jenkinson (1994) demonstrated that when *F. avenaceum*, *F. culmorum*, *F. poae* and *M. nivale* were grown in the presence of pollen of four wheat cultivars, 70-97% of the conidia germinated in 24 hours. Whilst in the absence of pollen, 40-50% of conidia of the same fungi germinated in 24 h. Studying the relationship between wheat anthers of different cultivars and floret infection by *Gibberella zeae*, Takegami (1957; 1958) suggested that cultivars which have short anther filaments, caught between the tips of closing glumes, were more susceptible to infection at early stages of flowering. Cultivars with anthers imprisoned by the glumes and eventually pushed out by developing grains were found to be more susceptible to late infection by *G. zeae*. Strange and Smith (1971) provided convincing evidence that anthers play an important role in the infection of wheat ears by *F. graminearum*. Following inoculation with conidia of *F. graminearum*, these workers observed significant infection in non-emasculated spikelets, whilst emasculated spikelets rarely became infected. They also observed that anther extract had a stimulatory effect on the growth of *F. graminearum* suggesting the presence of fungal growth stimulants. Further studies by Strange *et al.* (1974) identified betaine and choline as possible stimulatory compounds, which were isolated from wheat anthers. Work of Wiebe *et al.* (1989) confirmed the stimulatory effect of choline on the growth of two strains of *F. graminearum*. Recent work by Hilton (1999) showed that anthers have stimulatory effect on preliminary growth, and branching of conidial germ-tubes of *F. culmorum*. He suggested that this is probably not only due to betaine and choline content in pollen but also due to other unknown factors, which needs further investigation.

Andersen (1948) demonstrated that air temperature and moisture play an important role in the development of FHB. Following artificial inoculation of ears with *Gibberella zeae*, wheat plants

were incubated under temperatures ranging from 15–30°C and continuous wetness periods ranging from 6–72 hours. Most severe infection (96% spikelets infected) was observed in plants incubated at 25°C following 72 of continuous wetness whilst at 20 and 30°C, severity of FHB was 81% and 86% spikelets infected, respectively. Plants exposed to a wetness period of 48 hours exhibited 77% spikelets infected when incubated at 25°C and only 5% and 27% spikelets infected when incubated at 20°C and 30°C, respectively. At 15°C, infection of spikelets was negligible.

Jenkinson (1994) also demonstrated the importance of temperature on infection of wheat ears by *Fusarium* species. Following artificial inoculation of ears with conidial suspensions of either *F. avenaceum*, *F. culmorum*, *F. poae* or *M. nivale* wheat plants were incubated at temperatures ranging from 12 to 35°C at 90% relative humidity. Results showed that optimum infection of ears by *M. nivale* occurred at 15°C whilst optimum infection by *F. culmorum* occurred at 24°C. For both *F. avenaceum* and *F. poae* optimum infection was observed to occur at 25°C.

Teich (1989) reported that DON production in wheat heads inoculated with *F. graminearum* began about three days after infection and peaked about six weeks later before reducing to a constant level at plant maturity before harvest. In contrast, glasshouse experiment by Evans *et al.* (2000) showed that in two barley varieties inoculated with *F. graminearum*, DON and 15-ADON were detected in spikelets 48 h after inoculation. DON concentration increased after 72 h post-inoculation and did not diminish thereafter. 15-ADON peaked between 72 and 120 h post-inoculation and declined by 240 hours. Lacey *et al.* (1999) demonstrated that DON concentration in wheat grain was dependent on the growth stage at which plants became infected and on the duration of wet periods. In field plots, the highest DON concentration (12.12 mg kg^{-1}) in grain was detected when plants were inoculated with *F. culmorum* between GS 61-69 (flowering). Increasing the duration of wetness periods resulted in concurrent increases in DON contamination of harvested grain. Such observations agree with those of Prom *et al.* (1999) who also observed that the time of host infection and duration of wetness period influenced the amount of DON quantified in barley. DON concentration in barley grain in 1995 increased from 4.9 mg kg^{-1}at heading through 9.0 mg kg^{-1}at early milk to 36.8 mg kg^{-1} at late milk and then was relatively constant to the maturity of barley.

Since the effect of plant growth stage and duration of wetness periods on FHB severity and DON production is so similar, it could be suggested that a strong relationship exists between mycotoxin production and severity of symptoms observed. Indeed it could be speculated that mycotoxins may play an important role in determining pathogenicity of *Fusarium* spp. Further work is, however, required if such a relationship between toxin production and pathogenicity is to be demonstrated.

1.8 Control of FHB

A range of cultural, chemical and biocontrol strategies along with exploitation of resistant varieties can be employed in order to reduce the incidence and severity of FHB and mycotoxins in cereal crops.

1.8.1 Cultural control

1.8.1.1 Crop rotation

In order to reduce the risk of FHB epidemics, several cultural control techniques could be employed, including suitable crop rotation, appropriate use of fertilisers and weed control. Crop rotation is one of the most effective cultural control measures that can be adopted to reduce epidemics of FHB. Surveys involving 28 wheat crops grown in Illinois and Indiana (Holbert *et al.*, 1919) showed that when wheat crops were sown following maize, 15% became affected by FHB. However, when wheat was grown following either alfalfa or oats, only 4% of crops became infected. Similar observations were later recorded by Koehler *et al.* (1924) who studied the development of FHB in fields under different rotations. Where wheat was grown after maize, up to 43% of ears were observed to be affected by FHB. In wheat crops grown following rye or oats, 27 and 22.7% of ears showed symptoms of FHB, respectively, whilst following clover or timothy, only 11.4 and 8.3% of infected ears, respectively, was observed. Teich and Nelson (1984) observed that the average incidence of wheat crops affected by FHB on 72 farms in Southwestern Ontario was six times greater when wheat followed maize than when wheat crops followed soybeans or cereals. Recent experimental work by Dill-Macky and Jones (2000) on the effect of previous crop

residues and FHB development also demonstrated that the incidence and severity of FHB was greater when wheat followed corn and lower when wheat followed soybeans.

1.8.1.2 Land preparation

Removing or burying crop residues leads to a reduced source of FHB inoculum. Teich (1989) reported that in maize – wheat rotations, when wheat was planted after plots were ploughed, only 99 scabbed heads per 10^5 were observed, whilst in disc-cultivated plots, 209 wheat heads out of 10^5 showed symptoms. The author suggested that such observations were due to the greater quantity of corn residue left on the soil surface. Other workers have also demonstrated that removal or ploughing in of crop debris reduces the incidence of FHB in cereals. Studies by Obst *et al.* (1997) showed that wheat following grain maize had 0.5 mg kg^{-1} DON in comparison to that found in wheat following forage maize (0.3 mg kg^{-1}), which was probably due to a lower amount of crop debris remaining on the field after forage maize. In a three year field study on the effect of tillage practices on FHB in wheat, Miller *et al.* (1998) isolated *F. graminearum* from 79%, 55% and 46% of kernels in year one, two and three, respectively, in no-till plots, whilst the incidence of infected kernels was 20%, 40% and 13% in the three years when plots were tilled.

1.8.1.3 Nitrogen Inputs

Martin *et al.* (1991) during field trials on the effect of nitrogen inputs on the development of FHB, observed that increasing nitrogen applications from 70 kgN ha^{-1} to 170 kgN ha^{-1} resulted in concurrent increases in the incidence of *Fusarium*-infected grain in wheat, barley and triticale. Teich (1987) demonstrated that the use of different nitrogen sources applied to wheat might affect the incidence of wheat ears affected by FHB. In 1985 in Lambton County, Ontario, Canada, a comparative study showed that applications of ammonium nitrate resulted in 79 heads with FHB symptoms per 10^5 whilst applications of urea resulted in 59 bleached heads per 10^5. Yi *et al.* (2001) also demonstrated that applications of nitrolime to wheat plots could reduce the incidence of FHB by 59% when compared to plots treated with calcium ammonium nitrate. However, there was no significant effect on DON concentration in harvested grain. It is not clear how nitrogen affects FHB development. However, several hypotheses could be postulated including nitrogen influencing the

water potential of the plant which in turn could influence the susceptibility of ears to infection by *Fusarium* species. Further work is, therefore, necessary in order to determine the effect of nitrogen inputs on plant water potential, on the production of inoculum on infected stem bases and on the susceptibility of ears to infection by FHB causing pathogens.

1.8.1.4 Weed infestation

Field surveys in Southwestern Ontario have shown that fields with high weed densities had twice as many heads with FHB symptoms compared to weed-free fields (Teich and Nelson, 1984). The potential significance of weeds in the development of FHB epidemics has also been demonstrated by Jenkinson and Parry (1994) who isolated *Fusarium* species from 14 species of common broad-leaved weeds, that proved to be pathogenic to wheat. These workers suggested that weeds may provide an alternative source of inoculum for FHB epidemics and that weed control could prove useful for the reduction of inoculum.

1.8.2 Biological Control

Although there is limited information on the control of FHB by biocontrol agents, recent reports demonstrate that biocontrol of FHB pathogens has potential. For example, Bujold *et al.* (2001) showed that field antagonists of *G. zeae* may have an effect on the production of perithecia and ascospores of this pathogen. *In vitro* studies on wheat and maize residues (straw/stalk and grain) showed that inoculating residues with a *Microsphaerosis* species (isolate P130A) significantly reduced G. *zeae* ascospore production by 73%. When applied to crop residues in the field, the *Microsphaerosis* species had no effect on the pattern of perithecia formation, but significantly reduced perithecia production.

Bacterial biocontrol agents have also been investigated. For example, during two glasshouse studies, Khan *et al.* (1999) observed that applications of the bacterial strain AS 43.4 (*Bacillus* spp.) decreased disease severity of FHB by 67-95% and DON concentration in grain by 89-97%.

1.8.3 Genetic Resistance

There is a wealth of information on screening and breeding work aimed to identify sources of FHB resistance in wheat. As a result of a number of extensive studies, several types of host resistance mechanisms against FHB have been identified. Early studies by Schroeder and Christensen (1963) suggested that wheat scab resistance consist of at least two types of resistance: Type I resistance, which is resistance to initial infection and Type II resistance, which is resistance to fungal colonisation within a spike. Since this work, further studies have resulted in the proposition of two other resistance mechanisms; Type III resistance which is based on the ability of the host plant to degrade DON (Miller and Arnison, 1986) and Type IV resistance which is the tolerance of the host to high DON concentrations (Wang and Miller, 1988). Genetic resistance of cereals to FHB has been extensively reviewed by Arseniuk (1999), Bai *et al.* (2000), Buerstmayr, (1999), Hilton (1999), Mesterhazy, (1995), Mesterhazy *et al.* (1999), and Snijders (1990, 1994).

1.8.4. Chemical control

Effective chemical control against FHB pathogens *in vitro* (Table 1.1) and under field conditions (Table 1.2) has proved inconsistent. Moss and Frank (1985), for example, studied the effect of various concentrations of the fungicide tridemorph on T-2 toxin and diacetoxysciprenol (DAS) production by *Fusarium sporotrichioides in vitro*. At low concentrations (6-8 mg kg^{-1}) tridemorph caused slight enhancement of fungal growth whilst significantly reducing the production of T-2 toxin and DAS. At high concentrations (30-50 mg kg^{-1}) whilst fungal growth was inhibited by ca. 50%, T-2 toxin production was stimulated five-fold. Placinta *et al.* (1996) demostrated that a combination of both fungicide and prevailing temperatures influence fungal growth and toxin production by *F. sporotrichioides*. In a laboratory study, the fungus was placed on carbendazim-amended potato dextrose agar at concentrations ranging from 1.0 to 10 μg ml^{-1} and incubated at 25°C. After five days of incubation, half of the replicate plates were then incubated at 11°C. The results indicated that at 5 μg ml^{-1} carbendazim and 25°C, T-toxin production increased, however, no effect on zearalenone (ZEN) or neosolaniol (NEO) production was observed. Conversely, following the 25/11°C temperature regime, dose related inhibition of ZEN and T-2 toxin was

Table 1.1 Effect of fungicides on growth and mycotoxin production of FHB causing fungi *in vitro*.

Active ingredient	Details of tests *In vitro* studies	Reference	Notes
Tridemorph	Shake-flask cultures of *F. sporotrichioides*, fungicide added at 6µg and 36 µg ml^{-1}.	Moss and Frank (1985)	At 6µg growth enhanced but T-2 toxin and DAS inhibited at 36 µg ml^{-1}, growth inhibited but T-2 toxin production stimulated.
Hexaconazole and carbendazim	Culture of *F. culmorum* grown on amended PDA.	Waller *et al.* (1990)	Decreased growth of *F. culmorum*.
Dichloran, iprodione, vinclozolin	Added to potato-dextrose broth at 10, 100, 250 and 500 µg ml^{-1}. Static culture of *F. graminearum*.	Hasan (1993)	Dose related inhibition of growth and DAS and ZEN production. High concentrations completely inhibited DAS and ZEN production.
Carbendazim	Cultures of *F. sporotrichioides* on PDA, kept at 25°C or 25/11°C (diurnal). Fungicide added to PDA at 1, 2.5, 5, 7.5 and 10 µg ml^{-1}.	Placinta *et al.* (1996)	At 25°C and 5 µg ml^{-1} T-2 toxin production increased. At 25-11°C carbendazim significantly reduced T-2 toxin.
Difenconazole	Cultures of *F. culmorum* (sensitive (CS) and insensitive strain (IS) on PDA, at 25°C. Fungicides added at 0.1, 100 and 200 µg ml^{-1}.	D'Mello *et al.* (1997)	Progressive growth reduction.3 - ADON) production of (CS) was ceased at 100 and 200 µg ml^{-1}.
Prochloraz, tebuconazole, fluquinconazole, benomyl, carbendazim, thiabendazole, guazatine,	Culture of *F. graminearum* (Strain 4528) in liquid media. Chemical compounds added at various concentrations.	Matties *et al.* (1999)	Prochloraz and carbendazim inhibited growth and 3-ADON production. Tebuconazole and thiabendazole reduced growth but enhanced mycotoxin production at low levels. Benomyl increased fungal growth, but reduced 3-ADON.
Azoxystrobin	Shake-culture of *F. sporotrichioides* (carbendazim resistant strain) and control strain (CS) at 25°C.	D'Mello *et al.* (2001)	Suppressed mycelial growth, T-2 toxin enhancement at low levels of azoxystrobin (1 mg l^{-1}).
Tebuconazole	Culture of *F. culmorum* grown on PDA amended with 20 µg ml^{-1} of fungicide.	Kang *et al.* (2001)	Growth inhibition with marked morphological and cytological changes. No mycotoxin data.
Metconazole	Culture of *F. culmorum* grown on PDA amended with 20 µg ml^{-1} of fungicide.	Kang *et al.* (2001)	Fungal growth markedly retarded by fungicide. No mycotoxin data.

Table 1.2 Effect of fungicides on of FHB and mycotoxin production *in planta*.

Active ingredient	Details of tests *In planta* studies	Reference	Notes
Benomyl	Field trail with natural infection.	Jacobsen (1977)	Reduced grain infection from 24% in untreated plots to 4-8% in treated ones.
Propiconazole	Field trial with artificial inoculation.	Martin and Johnston (1982)	A 41% decrease of FHB severity, no effect of DON. Yield increased by34%.
Benomyl, thiabendazole	Field trial with artificial inoculation.	Carranza (1988)	A 87% decrease of seed infection by *Fusarium* spp.
Thiophanate methyl	Field trial with natural infection.	Ueda and Yoshizawa (1988)	Up to 100% DON reduction in grain.
Fenpropimorph, prochloraz, propiconazole	Trials with natural infection. Fungicides applied at various growth stages.	Michail (1989)	Negligible effect on seed infection with *Fusarium* spp.
Triadimefon, propiconazole, thiabendazole	Field trial with artificial inoculation of *F. graminearum*.	Boyacioglu *et al.* (1991)	Reduction of DON and FHB severity by triadimefon, propiconazole up to 61% and79% respectively. Maximum DON reduction of 83% by thiabendazole, no effect of grain infection.
Prochloraz, tebuconazole, triadimenol	Glasshouse trial with artificial inoculation with *F. avenaceum*, *F. culmorum*, *F. graminearum* and *M. nivale*.	Hutcheon and Jordan (1992)	Effective post inoculation control against all species.
Tebuconazole, triadimenol	Field trail with natural infection.	Wainwright *et al.*(1992)	Decrease spore counts of *F. culmorum*.
Benomyl, tebuconazole, propiconazole, thiabendazole	Field trial with artificial inoculation with *F. graminearum*.	Milus and Parsons (1994)	No effect of FHB severity, DON concentration in grain and on yield.
Tebuconazole+triadimenol	Field trial with artificial inoculation with *F. culmorum*.	Gareis and Ceynowa (1994)	Decreased FHB by 64%, DON content decreased but nivalenol (NIV) increased 16 or 6 fold, depending on time of fungicide application.
Tebuconazole, tebuconazole+triadimenol, prochloraz	Field trial with artificial inoculation with *F. culmorum*.	Ellner (1997)	Decreased of DON by 70% and FHB severity by 65%.
Tebuconazole	Field trial with artificial inoculation with *F. culmorum*.	Homdork *et al.* (2000)	FHB severity decreased by 91.8%, DON reduced by 79.8%. No NIV detected.

Table 1.2 (Continued)

Active ingredient	Details of tests	Reference	Notes
Tebuconazole, benomyl, propiconazole, azoxystrobin	Field trial with artificial inoculation with *F. graminearum.*	Jones (2000)	All fungicides reduced FHB severity by at least 56%, DON reduced by at least 34%. Azoxystrobin had negligible effect.
Tebuconazole, azoxystrobin, fluquinconazole	Field trial with artificial inoculation with mixture of *F. avenaceum, F. culmorum,* and *M. nivale.*	Simpson *et al.* (2001)	Tebuconazole reduced DON by 56%; about two fold increase of DON production by azoxystrobin and fluquinconazole compared to untreated control.
Tebuconazole, metconazole, prochloraz, benomyl	Field trial with artificial inoculation with *F. culmorum.*	Siranidou and Buchenauer (2001)	Disease severity reduced by 89% by tebuconazole and metconazole, DON reduced by 70%. Azoxystrobin enhanced DON production; benomyl and prochloraz had no effect on FHB and DON.
Tebuconazole, carbendazim, azoxystrobin	Field trail with natural infection where *F. graminearum* was the most common FHB pathogen.	Cromey *et al.* (2001)	FHB reduced by 47%; Tebuconazole and carbendazim reduced NIV and DON by 67% and 70% respectively. Azoxystrobin had no effect on the mycotoxin production.

observed. One possible explanation for these variable reactions of *F. sporotrichioides* is the existence of carbendazim resistant strains within the species. D'Mello *et al.* (2000) demonstrated that when two strains of *F. sporotrichioides* - control strain (CS) and resistant to carbendazim strain (RS) were grown at 25°C in a peptone broth containing 1, 2, or 4 μg ml^{-1} carbendazim, the CS isolate showed dose related effects in inhibition of fungal growth and T-2 toxin production, whilst 2 μg ml^{-1} addition enhanced T-toxin production by the RS isolate, with no effect on the mycelial mass.

In vitro work determining the efficacy of fungicides against *Fusarium* spp. by Hasan (1993) showed that dichloran, iprodione and vinclozolin were effective against *F. graminearum.* All three fungicides, when added to potato-dextrose broth at 10, 100, 150 and 500 μg ml^{-1}, provided significant reduction of mycelial mass, DAS and ZEN production. Dichloran eliminated DAS production at 500 μg ml^{-1} and sufficiently eliminate ZEN production at 250 μg ml^{-1}. Iprodione markedly reduced DAS production at 100 μg ml^{-1} and prevented it at 250 μg ml^{-1}. Vinclozolin prevented DAS production at 250 μg ml^{-1} and at 500 μg ml^{-1}, significantly reduced ZEN. In contrast, recent work by Matthies *et al.* (1999) demonstrated that *F. graminearum* could react in various ways when incubated in the presence of sub-lethal concentrations of some chemical compounds such as prochloraz, tebuconazole, benomyl, carbendazim and thiabendazole. In a series of trials, these workers showed that prochloraz effectively inhibited both mycelium growth of *F. graminearum* and 3-ADON production to the same extent. Tebuconazole inhibited fungal growth at each of the concentrations 0.1, 0.5 and 1 μg ml^{-1}. However, at 0.51 μg ml^{-1}, 3-ADON production was increased four-fold when compared to control treatments. At 0.1 μg ml^{-1}, benomyl increased mycelium growth of *F. graminearum* by 22% and diminished 3-ADON production by 22% compared to untreated control. Dose-related inhibition of mycelial growth and mycotoxin production was observed when carbendazim was added to the media at 0.5, 0.7, 1, 1.5 and 2 μg ml^{-1}. When guazatine and iminoctadine were added to the growth media, both fungicides significantly reduced mycelium growth of *F. gramienarum* but markedly increased 3-ADON production by up to 200%.

Glasshouse and field trials, which have been conducted to assess the efficacy of fungicides against FHB, have yielded conflicting results. For example, Jacobsen (1977) studied the effect of benomyl (0.55 kg a.i ha^{-1}), mancozeb (1.76 kg a.i ha^{-1}), mancozeb plus benomyl (1.1 kg a.i ha^{-1} plus 0.27 kg a.i ha^{-1}) and benomyl plus carbendazim (0.55 kg a.i ha^{-1} plus 1.1 kg a.i ha^{-1}) on Septoria leaf and glume blotch and FHB caused by *F. graminearum* on grain yield and test weight of wheat. Fungicide applications resulted in an increase in test weights by up to 2% over unsprayed control plots for benomyl, 1.2% for the mixture benomyl+mancoceb and 1.7% for mancoceb. All treatments reduced the percentage of scab infected grain by 50%. This is in agreement with the work of Carranza (1988) who showed that an application of benomyl and thiabendazole applied 5 days before expected flowering date reduced the incidence of wheat grain infected with *F. graminearum* by 88% and increased grain germination by 65% and 1000-grain weight by 48%. In contrast, Michail (1989), who observed the incidence of *Fusarium*-infected seed in 32 samples of 16 wheat varieties from crops sprayed with a range of fungicides (triadimefon, captfol+triadimefon, fenpropimorph, carbendazim, propiconazole, captafol+pyrazofos and prochloraz) at growth stages between GS 32 and GS 50 (Zadoks *et al.*, 1974) failed to find any significant effect of the fungicide treatments on grain infection.

Huthceon and Jordan (1990) during an evaluation of fungicides against different FHB pathogens under glasshouse conditions, demonstrated that carbendazim, prochloraz and propiconazole were effective against *F. avenaceum*, *F. culmorum* and *F. graminearum* by reducing them in wheat spikelets by 70% over the control treatment. Studies in naturally infected trials in the Atlantic Provinces in Canada by Martin and Johnston (1982) showed that propiconazole at a rate of 250g a.i. ha^{-1} applied at GS 51 and GS 73 provided good control of FHB and increased yield by 34% compared to the control. They did not observe any effect of the fungicide treatments on the concentration of DON in grain. Milus and Parsons (1994) studied the effect of benomyl, chlorothalonil, fenbuconazole, flusilazole, myclobutanil, potassium bicarbonate, propiconazole, tebuconazole, thiabendazole and triadimefon plus mancozeb on FHB severity, DON contamination and yield of winter wheat. During two years of field studies, these authors did not observed any significant effect of these fungicides on FHB, yield and mycotoxin levels in harvested grain.

During a field trial where wheat plots were inoculated with *F. culmorum* before being sprayed with the fungicide Matador® (tebuconzole+tiabendazole) either 3 hours or 24 hours after inoculation, Gareis and Ceynova (1994) reported a 16-fold increase in nivalenol concentration in harvested grain when the fungicide was applied 3 hours post-inoculation and a six-fold increase when applied 24 hours post-inoculation. The severity of FHB symptoms was reduced by 54%. Although it is difficult to draw firm conclusions from just one year's work, such observations raise the question of how each of the trichothecene chemotypes (Miller *et al.*, 1991) produced by *Fusarium* spp. are affected by different fungicides.

During fungicide efficacy field trials in Hungary, Mesterhazy and Bartok (1996) applied triadimefon, carbendazim, bromuconazole, cyproconazole+carbendazim, propiconazole, tebuconazole+triadimenol, and tebuconazole to plots of the FHB susceptible wheat varieties Zombor and Csaba, one day after the artificial inoculation of ears with *F. graminearum* and *F. culmorum* at full flowering. Results obtained showed that those fungicide treatments, which included the triazole tebuconazole, reduced grain infection between 97-99% and DON contamination by 100%. Conversely, those treatments, which did not include tebuconazole only, reduced, grain infection by 43-87% and DON contamination by 50%. The work of Ellner (1997) also confirmed the efficacy of the 'azole' fungicides against FHB when applications of either tebuconazole, tebuconazole+triadimenol or prochloraz to ears of wheat artificially inoculated plots with *F. culmorum*, significantly reduced FHB severity by 50% and DON concentration in grain by 85%. One possible reason for the inconsistent control of FHB achieved by fungicides under field conditions could be due to the complex interaction, which may occur between fungicides, *Fusarium* species and other ear-colonising fungi such as *Alternaria* spp., *Septoria* spp, *Cladosporium* spp. and *Botrytis* spp. For example, Bateman (1979) studied the relationship between saprophytic ear colonising species and *M. nivale* on wheat ears and wheat seed. Grain collected from wheat ears which were artificially inoculated with either *Alternaria* spp., *Cladosporium* spp. or *Sporobolobomyces* spp. at anthesis prior to the inoculation of ears with *M. nivale*, yielded significantly less *M. nivale* in comparison with saprophyte-free ears. Similar results have been shown by Liggitt *et al.* (1997), during glasshouse studies where wheat plants were

inoculated with either, *A. alternata*, *B. cinerea* or *C. herbarum* at GS 59 prior to inoculation with *F. culmorum* at GS 65. The presence of any one of the three saprophytic species reduced FHB severity between 46% and 78% compared to plants inoculated only with *F. culmorum*. Furthermore, when each of the saprophytic species were introduced to ears after their inoculation with *F. culmorum*, *B. cinerea* and *C. herbarum* did not have any effect on FHB severity, whilst *A. alternata* significantly increased disease symptoms by 34% over the control treatment. Liggitt *et al.* (1997) also demonstrated during *in vitro* work, that fungicides had differential effect on the species used in their studies. For example, pyrimethanil reduced mycelial growth of *A. alternata* by up to 92%, but failed to reduce growth of *F. culmorum*, *B. cinerea* or *C. hernarum* by more than 27%. Conversely, flusiazole reduced mycelial growth of F. *culmorum* by up to 90% but failed to reduce mycelial growth of *B. cinerea* or *C. herbarum* by more than 59%. As a result of their observations, these workers suggested that the application of fungicides which have limited activity against *F. culmorum*, but which have significant activity against saprophytic species, may lead to greater colonisation of wheat ears by the pathogen, due to the removal of antagonistic saprophytes. However, further work is needed in order to determine if this is the case under field conditions.

Work by Jennings *et al.* (2000) has demonstrated that applications of the strobilurin fungicide, azoxystrobin, may have a significant effect on the interaction between *F. culmorum* and *M. nivale* colonising wheat ears. During two years of field studies, applications of either tebuconazole, metconazole or carbendazim resulted in a significant reduction in both DON concentration and the extent of grain colonisation by *Fusarium* spp. quantified by a competitive PCR assay. Conversely, applications of the same fungicides resulted in an increase in the extent of grain colonised by *M. nivale*. In the first year of the study, the effective reduction of *M. nivale* on wheat ears, achieved following applications of azoxystrobin, alleviated competition between *M. nivale* and *Fusarium* spp. As a result of this reduced competition, greater colonisation of ears by *Fusarium* spp. was observed and DON contamination was increased by 41%. In the second year, *M. nivale* was not present on wheat ears and as such, no significant increase of DON contamination of grain after treatment of azoxystrobin was detected. More recently, Simpson *et al.* (2001) have also associated applications of azoxystrobin with increased DON concentrations in harvested wheat grain. During

a field trial where wheat ears were artificially inoculated with a mixture of *F. avenaceum*, *F. culmorum* and *M. nivale* at GS 65 before being sprayed with an application of azoxystrobin three days later, these workers observed a 40% increase in DON concentration in harvested grain when compared to grain harvested from unsprayed plots. The quantification of *F. culmorum* DNA did not indicate an increase of this species in grain, although *M. nivale* DNA was significantly reduced by azoxystrobin. It is possible, therefore, that applications of the fungicide azoxystrobin may have a direct effect on DON production. However, further study is necessary to see what the effect of azoxystrobin could be when applied against FHB caused by *F. culmorum* or *F. graminearum* without *M. nivale* present under more controlled conditions.

Another possible reason for the inconsistent control of FHB achieved by fungicides under field conditions could be due to their time of application. Studies where fungicides have been applied between growth stage (GS) 32 and GS 50 have failed to reveal any significant reduction of FHB (Michail, 1989; Hutcheon and Jordan, 1992). However, studies in which fungicides were applied between GS 59 and 70 have demonstrated significant reduction in both the severity of FHB and mycotoxin concentration in harvested grain. For example, Boyacioglu *et al.* (1992), during a field trial where wheat plots were artificially inoculated with *F. graminearum* at anthesis, observed that the fungicide triadimefon was effective at reducing grain infection and DON concentration when applied two days pre-inoculation, at the time of inoculation and when applied 2 days post-inoculation of ears and reduced DON concentration and grain infection between 65-79%, but reduced disease severity by 50-62% at the second and third time of application, respectively. Propiconazole also reduced *F. graminearum* infection by 39-56% and DON concentration by 62-79% when applied at the time of inoculation and 2 days post-inoculation of ears, respectively. Conversely, thiabendazole proved most effective at reducing DON concentration when applied 2 days pre-inoculation by 83%, despite the fungicide having no effect on the incidence of grain infection. In Germany, Matthies and Buchenauer (2000) reported that applications of either tebuconazole or prochloraz, 2 days post-inoculation (GS 65) of wheat inoculated with *F. culmorum*, reduced disease severity by 56% and 41%, respectively, whilst 8 days pre-inoculation and 9 post-inoculation were less effective. Tebuconazole and prochloraz applied at 2 days post-

inoculation diminished DON content in grain by 43% and 22%, respectively. Contrary to this Homdork *et al.* (2000b) found that an application of tebuconazole against FHB (*F. culmorum*) three days pre-inoculation reduced disease severity by 92% and DON concentration in grain by 68.8 % in comparison with control treatment, whilst the same fungicide applied 5 days post inoculation reduced FHB by 57% and DON in grain by 53.5%. In this study, tebuconazole was also applied twice pre- and post-inoculation and provided the highest reduction of DON in grain by 79.8% and reduced FHB severity by 90%. Suty *et al.* (1996), also reported double treatment of tebuconazole at GS 55 and 69 to be the most efficacious treatment compared to single treatments at either GS 55 or 69, however this would be impractical to many growers and would result in increased fungicide input.

More recently, Siranidou and Buchenauer (2001) showed that applications of tebuconazole two days before and two days post-inoculation of wheat plots with *F. culmorum* reduced severity of FHB and DON content in wheat grain by 61-89% and 50-70%, respectively. Metconazole was applied at only two days pre-inoculation and reduced DON and FHB severity by 69% and 71% respectively. Chlorothalonil, prochloraz and benomyl failed to effectively control FHB.

In contrast to the observations of Jennings *et al.* (2000) and Simpson *et al.* (2001), Jones (2000) applied azoxystrobin during field trials inoculated with *F. graminearum* between 1995 and 1997, and observed a decrease in FHB severity by 12% compared to unsprayed controls and reduction of DON by 25%. Siranidou and Buchenauer (2001) have also found that applications of azoxystrobin against FHB caused by *F. culmorum* reduced disease severity significantly. However, the DON concentration in grain remained unchanged when compared to unsprayed controls. Furthermore, Cromey *et al.* (2001) demonstrated that azoxystrobin could provide some control of FHB, without any detrimental effects on the mycotoxin production. Azoxystrobin, tebuconazole and carbendazim were applied at GS 59 or 65 on winter wheat plots naturally infected with *Fusarium* spp. (most predominant *F. graminearum*). Tebuconazole reduced disease severity by 41%, whilst azoxystrobin and carbendazim reduced FHB by 29% compared to control treatment. Tebuconazole

and carbendazim significantly reduced DON and NIV in grain, whilst azoxystrobin did not have any effect on the investigated mycotoxins.

Aims of the Project

1. *To determine the effect of fungicide applications on the FHB severity, yield and colonisation and accumulation of deoxynivalenol in grain of winter wheat by Fusarium spp. and Microdochium nivale*

This was achieved by conducting a field trial where the fungicides azoxystrobin, metconazole or tebuconazole were applied at GS 59 at either full or half the manufacturer's recommended dose rate to wheat plots inoculated with a conidial suspension (10^5 spores per ml^{-1} of water) of *F. graminearum*, *F. culmorum* and *M. nivale* (1:1:1 ratio). In addition, two glasshouse experiments were undertaken to determine if the fungicides metconazole and azoxystrobin applied at GS 59 at double, full, half and quarter manufacturers recommended dose rate to plants inoculated with conidia of either *F. graminearum* or *F. culmorum* at GS 65 result in elevated concentration of DON in grain.

2. *To evaluate the effect of timing of fungicide applications on symptom development of Fusarium head blight caused by range of head blight pathogens, yield of winter wheat and accumulation of deoxynivalenol in grain*

The effect of different timing of fungicide applications on Fusarium head blight (FHB) severity and mycotoxin accumulation in wheat grain was investigated during two field trials. One field trial was conducted to investigate if fungicides metconazole, azoxystrobin, fluquinconazole, or mixture of metconazole and azoxystrobin at full manufacturer's recommended dose rate applied early in the growing season (GS 31+39) can provide significant control of FHB mycotoxin accumulation in wheat in comparison with the effect of the same fungicides applied later in the season (39+59). The second field trial involved the fungicides metconazole, tebuconazole, azoxystrobin and mixtures of metconazole+azoxystrobin and tebuconazole+azoxystrobin which were applied at half the manufacturer's recommended maximum dose rate, at either 5 days pre-, 2 days pre-, 2 days post- or 5 days post-inoculation of wheat plots with *Fusarium spp.* and *Microdochium nivale* at GS 65.

3. *To determine the role of saprophytic species on the colonising wheat ears on the development and fungicidal control of Fusarium head blight and the subsequent production of deoxynivalenol in wheat*

In three glasshouse experiments, the effect of metconazole and azoxystrobin on the interactions between *Fusarium culmorum* and *M. nivale* and also between *F. culmorum* and *Alternaria tenuissima* or *Cladosporium herbarum* and the development of Fusarium Head Blight and deoxynivalenol (DON) production were studied. Plants were inoculated with *F. culmorum* at GS 65, whilst *A. alternata*, *C. herbarum* and *M. nivale* were inoculated either at GS 57 or 24 hours after introduction of *F. culmorum* on the wheat eras alone or in combination with fungicide metconazole or azoxystrobin applied at GS 59 at full manufacturer's recommended dose rate.

Chapter 2

General Materials and Methods

2.1 Pathogens

Table 2.1 lists the fungal isolates used during the experimental work undertaken in this project. *Fusarium* isolates were obtained from the Central Science Laboratory, York, UK courtesy of Dr Phil Jennings and from the culture collection of Harper Adams University College, Shropshire, UK. *Microdochium nivale* isolates were obtained from the Harper Adams University College culture collection. Isolates of *Alternaria tenuissima* and *Cladosporium herbarum* were obtained from Cranfield Biotechnology Centre, Cranfield University, Cranfield, Bedfordshire, UK courtesy of Professor Naresh Magan.

Table 2.1 Isolates used in the experimental work

Species	Isolate	Origin
Fusarium culmorum (W.G. Smith) Sacc.	Fc 47/1, F 95 Fc 53, Fc 70	Harper Adams University College Central Science Laboratory, York
Fusarium graminearum Schwabe	Fg F98, Fg NFTP Fg 113, F145	Harper Adams University College Central Science Laboratory, York
Microdochium nivale Samuel and Hallet	1/1, 30/1, 74/1, 80/1 94/1, 117/1	Harper Adams University College
Alternaria tenuissima *Cladosporium herbarum* (Pers)		Cranfield Biotechnology Centre Cranfield Biotechnology Centre

2.2 Culture and storage of pathogens

All aseptic operations were carried out in a sterile laminar flow cabinet. Glassware, media and sterile distilled water were autoclaved at 121°C and 103.4 KPa for 20 minutes.

Fungal isolates obtained from Central Science Laboratory, York and Cranfield Biotechnology Centre were received as actively growing cultures on artificial media. Isolates from Harper Adams University College were taken from the pathogen collection and maintained as a spore suspension in 10% glycerol at –80°C. All isolates were sub-cultured onto Potato Dextrose Agar (PDA) (Lab M, Bury, UK) and sealed with Parafilm M (American Can Company, Chicago, USA). After two weeks in an incubator at 20°C +/- 2°C, cultures were transferred to the refrigerator for storage at 4°C. All isolates were sub-cultured after three months storage onto fresh media in order to maintain pathogenicity.

2.3 Spore production

Sub-cultures of each isolate were produced by taking 5mm diameter plugs of inoculum from the edges of actively growing cultures using a sterile cork borer and transferring them onto plates of PDA. Petri dishes then were incubated under darkness at 20°C+/- 2°C for 14 days. In order to induce sporulation, 14-day-old cultures were then placed in an incubator under continuous near-UV light for a further 7-14 days at 20°C+/- 2°C.

2.4 Preparation of spore suspensions for experimental use

Conidial suspensions were obtained for each isolate by washing conidia from sporulating colonies using sterile distilled water. A sterile spatula was used to assist in the dislodging of conidia and mycelium from the agar. The suspension obtained was then filtered through two layers of sterile muslin to remove hyphal fragments. Spore concentration was then determined using a haemocytometer (Weber Scientific International Ltd, Teddington, Middlesex, UK) and adjusted to the required concentration.

2.5 Production of experimental plants for greenhouse studies

Fungicide treated seed (fludioxinil at 25 g. a.i. per 100 kg of seed, Beret Gold®, Syngenta Crop Protection) of the winter wheat cv. Cadenza was sown into 15 cm diameter plastic pots containing John Innes Number 2 compost at a rate of five seeds per pot. Once seedlings had emerged, plants were placed in the bay of a glasshouse set at 10 ± 3°C under a photoperiod of 12 h for 30 days in order to encourage efficient tillering. After 30 days, the temperature was increased to 22 ± 3°C and the photoperiod to 16 h. Plants were watered daily and fed once a week with an application of foliar fertiliser (10% N, 10% P_2O_5, 27% K_2O as Phostrogen®, Phostrogen Ltd, Corwen, Clwyd, UK). When necessary, plants were sprayed with the fungicide Fortress® (500 g a.i l^{-1} quinoxifen, Dow AgroSciences) at a rate of 0.3 l ha^{-1} to prevent infection by powdery mildew. Nicotine shreds (Nicotine 40% Shreds®, Dow AgroSciences) were also used according to the manufacturer's recommendation to fumigate the glasshouse when necessary to eradicate aphid infestations.

2.6 Artificial inoculation of wheat plants

During glasshouse experiments, artificial inoculation was achieved by spraying prepared conidial suspension onto ears using a hand-held atomiser until run-off. This equated to approximately 2.5 ml of spore suspension per ear. In order to create conditions conducive for infection by the pathogens, all inoculated ears were immediately covered with polythene bags, which were then removed 48 h later. Under field conditions, plots of wheat were artificially inoculated using a hand pressurised knapsack sprayer (Bastion 15, Application technique Ltd, Herts, UK) with a conidial suspension at rate 33 ml m^{-2}. In order to produce conditions conducive to FHB, immediately after inoculation, overhead mist-irrigation was applied to plots for 60 seconds every 20 minutes between 08.00 and 18.00 hours. Mist irrigation was continued for 21 days following inoculation. (Plate 2.1).

2.7 Fungicide application

During glasshouse studies, all fungicide treatments were applied using a precision pot sprayer (custom built for Harper Adams University College by J.Reader) carrying Lurmark 110° flat fan nozzles (03-F110, Longstanton, Cambridge, UK) at a rate of 200 l ha^{-1}. During field trials, fungicide applications were made using a pressurised knapsack sprayer (Safer spa, Italy) with a four-nozzle boom. Fungicides were applied at three bar pressure, at 200 l of water per ha^{-1} using Lurmark 110° flat fan nozzles. The fungicides tested during field and glasshouse experiments are listed in Table 2.2.

Table 2.2 Fungicides used in field and glasshouse studies.

Active Ingredient	Concentration	Product Name	Manufacturer's recommended rate	Manufacturer
Azoxystrobin	250 g a.i. l^{-1}	Amistar	1 l ha^{-1}	Syngenta
Fluquinconazole	100 g a.i. l^{-1}	Flamenco	1.5 l ha^{-1}	Aventis CropScience UK Ltd
Metconazole	60 g a.i. l^{-1}	Caramba	1.5 l ha^{-1}	BASF Plc
Tebuconazole	250 g a.i. l^{-1}	Folicur	1 l ha^{-1}	Bayer Plc

a

b

Plate 2.1 Overhead mist-irrigation of plots of winter wheat

2.8 Disease Assessment

In both field and glasshouse studies, the incidence of FHB (expressed as the percentage of ears infected) and severity of FEB (expressed as the percentage of spikelets infected) was recorded at GS 85. The severity of sooty mould on wheat ears was assessed at GS 90 using a 1-9 scale where 1 = healthy ears and 9 = all ears completely covered in black sooty spores.

2.9 Yield assessment

When ripe (GS 92), all field plots were individually harvested using a Seedmaster Plot combine (Wintersteiger, Ried im Innkreis, Austria). During the harvesting operation, total grain yield and grain moisture content was recorded. Two-kilogram grain samples were taken from each plot and used to determine grain specific weight and thousand grain weight. From each two kilogram grain sample, a sub-sample of approximately 230 g was also taken using a ripple grain divider (Novaliter Laboratories, UK) and used for the quantification of grain colonisation by *Fusarium* spp. and *M. nivale* and for DON analysis.

2.10 Visual assessment of *Fusarium* infected grain

Two hundred grains selected at random from each field plot were obtained and visually assessed. The incidence of grain, which appeared shrivelled or had a pink discoloration was recorded and expressed as a percentage of the total number of grains.

2.11 DNA extraction

A hammer was used to crush 14 g of grain within an envelope made from a heat-sealed A4 acetate sheet. DNA was extracted from crushed grain samples using a CTAB buffer (sorbitol 23 g, n-lauryl sarcosine 10 g, cetyltrimethylammonium bromide 8 g, sodium chloride 87.7 g and polyvinylpolypyrrolidone 10 g) per l^{-1}. Thirty ml of CTAB buffer was added to a 10 g sample of crushed grain in a 50-ml centrifuge tube, mixed, and incubated at 65°C for 16 h. Ten ml of potassium acetate (5M) was added, mixed, and tubes frozen for 1 h at -20°C. Tubes were thawed, mixed and centrifuged (3,000 x *g*, 15 min). A 1.3 ml aliquot of supernatant was removed and added to 0.6 ml chloroform in a 2-ml Eppendorf tube. Tubes were mixed by gentle inversion for 1

min then centrifuged (12,000 x *g*) for 15 min. A 1 ml aliquot of the aqueous phase was removed to a fresh tube containing 0.8 ml 100% isopropanol. Tubes were mixed by gentle inversion for 1 min, incubated at 18°C for 30 min then centrifuged (6000 x *g*, 15 min). Resulting DNA pellets were washed twice with 44% isopropanol then air dried. Pellets were re-suspended in 200 µl TE buffer (10 mM TRIS-HCl, 1 mM EDTA pH 8.0) at 65°C for 1 h before storing at 4°C. Total DNA was quantified by spectrophotometry using a Beckman 640 Spectrophotometer according to the manufacturer instruction (Beckman Instruments Inc., Fullerton, California) and diluted to a DNA concentration of 40 ng μl^{-1}, 4 ng μl^{-1} and 0.4 ng μl^{-1}.

2.12 Quantitative Polymerase Chain Reaction (PCR) for trichothecene producing *Fusarium* spp. identification

For each treatment, the *Tri*5 gene, which encodes trichodiene synthase, an enzyme which catalyses the initial reaction in the biosynthetic pathway of all trichothecene mycotoxins (Kimura *et al.*, 2001), was quantified using an assay developed by Dr S. Edwards (Personal communication). PCR mixtures (50 µl) contained 100 µM of each nucleotide, 100 nM of HATri/F [CAGATGGAGAACTGGATGGT] and HATri/R [GCACAAGTGCCACGTGAC], 20 units/ml Red Hot *Taq* polymerase (ABgene, Epsom, UK), 10 mM TRIS-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µg ml^{-1} gelatin, 0.5 mg ml^{-1} Tween 20 (Sigma), and 0.5 mg ml^{-1} Nonidet P-40 (Sigma) and 10 µl of DNA sample. Ten µl of *F. culmorum* standards (4 to 260 pg μl^{-1}) were also amplified with 10 µl HATriIS in the same experiment to produce a standard curve. Three controls were used; HATriIS alone, *F. culmorum* alone, and water. Amplification was performed in a PTC 100 thermal cycler (MJ Research, USA). The program used had 35 temperature cycles of 94°C for 15 s, 62°C for 15 s, and 72°C for 45 s. The first cycle had an extra 75 s at 94°C and the final cycle had an extra 4 min 15 s at 72°C. PCR products were observed after electrophoresis through agarose gels (2% w/v containing 0.5 µg ml^{-1} ethidium bromide) in TAE buffer (40 mM TRIS-acetate, 1 mM EDTA pH 8.0). Following gel electrophoresis, gels were viewed under UV light on a Gel Doc 1000 fluorescent gel documentation system (Bio-Rad Laboratories Ltd, Hemel Hempstead, UK) and unsaturated gel images were analysed using Molecular Analyst software (Bio-Rad). PCR product ratios were determined for each standard and sample by dividing the band

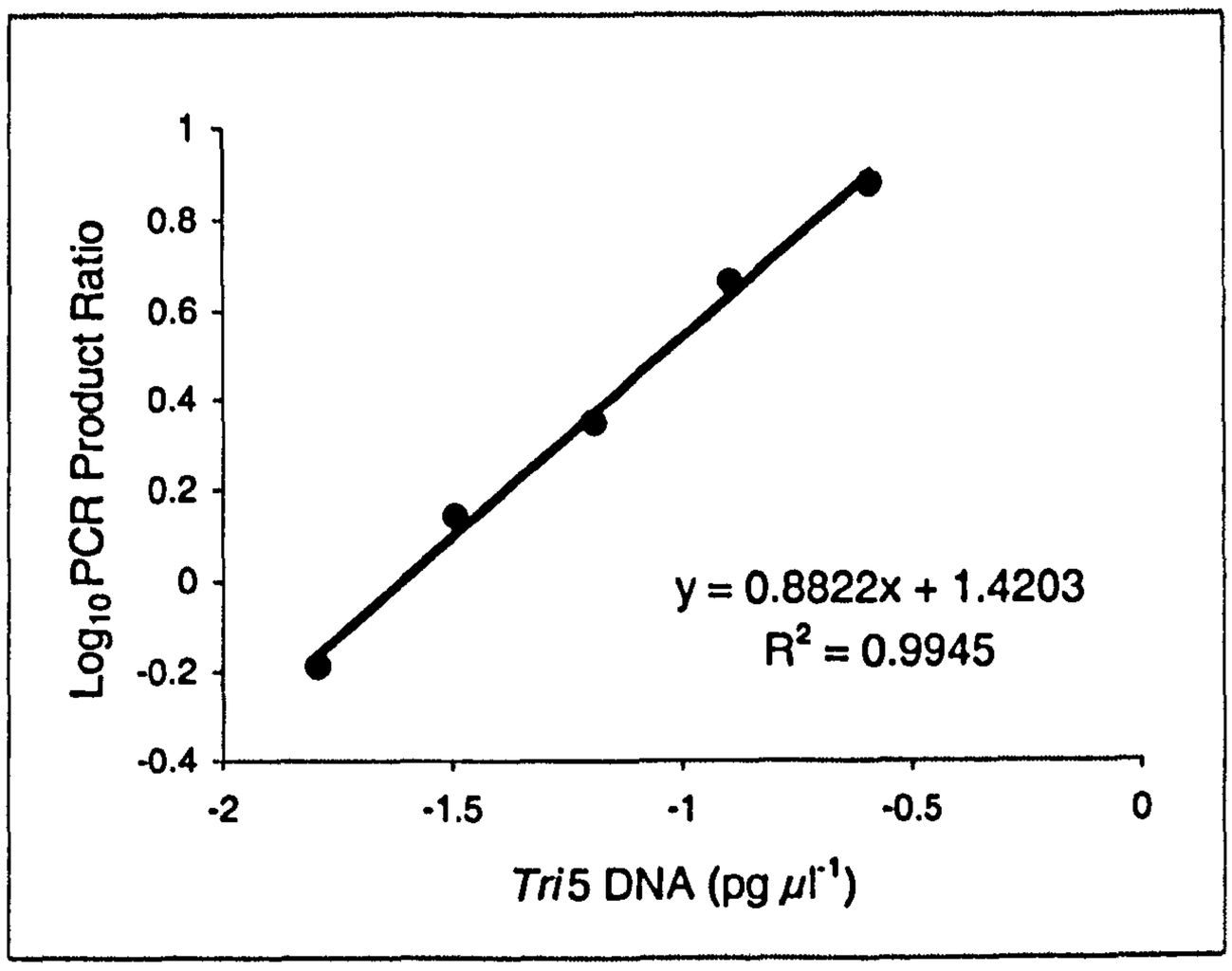
intensity of the *Tri*5 gene product (260 bp) by the HATriIS product (430 bp). As the standard curve was generated using genomic DNA of *F. culmorum* F36, then the unit of DNA quantified is the amount of *Tri*5 DNA present within a pg. of *F. culmorum* F36 genomic DNA. Figure 2.1a shows the first standard curve using this assay. All standard curves used in this thesis had a r^2>0.96.

Quantitative PCR for *M. nivale* identification

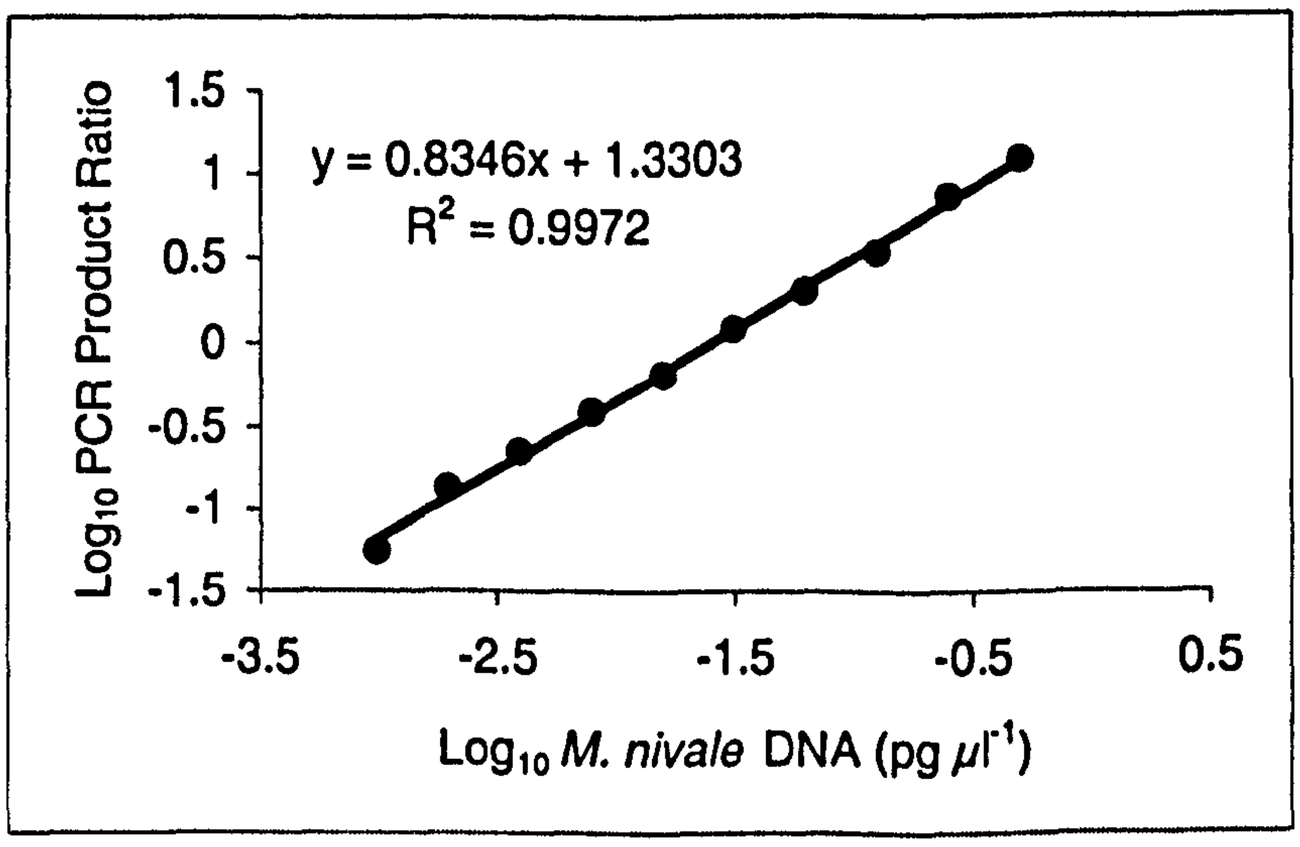
The presence of *M. nivale* in harvested grain was also quantified using competitive PCR The method was as described above for *Tri5* quantification except the PCR program had an anneal of 66°C, the primers were HAMn/F and HAMn/R, the internal standard was HAMnIS and the fungal standards used were a two-fold serial dilution of DNA from isolate, *M. nivale* 117M (1 to 500 pg μl^{-1}). Primers and internal standards for *Tri*5 and *M. nivale* quantification were developed by Dr S.Edwards at Harper Adams University College. Figure 2.1b shows the first standard curve using this assay. All standard curves used in this thesis had a r^2>0.98.

DON analysis

Deoxynivalenol concentration in grain was quantified using Ridascreen®DON Fast immunoassay (R-Biopharm Rhône Ltd, Glasgow, UK). Two hundred grams of grain per replicate were milled using a Falling Number Laboratory Mill 3100, Sweden. In order to avoid cross contamination the mill was cleaned after milling of each sample. Two grams of flour per sample was added to 50-ml centrifuge tubes then 40 ml of distilled water was added to the tubes. Tubes were shaken for three minutes and then left to settle for an hour. A 1-ml aliquot was removed to a 1.5-ml eppendorf tube, and then centrifuged (12000 x g, 15 min.). After adding sufficient number of wells into the microwell holder 50 µl of standards or prepared samples were pipetted to each well. Fifty microliters of enzyme conjugate and 50 µl of the DON antibody were added to the bottom of each well, mixed thoroughly by gently rocking the plate then incubated for 5 min (+/- 1) at room temperature (20-25°C). Samples were removed from the wells and wells washed three times using the wash buffer. One hundred µl of chromogen (substrate) was added to each well, and after mixing, was incubated for 3 min (+/- 0.5) in the dark at room temperature (20-25°C). After incubation, 100 µl of stop solution was added to each well. Samples were mixed well and read



a



b

Figure 2.1 Standard curves used for PCR quantification of *Tri5* DNA (a) and for *M. nivale* DNA (b) in harvested grain.

within 10 minutes using a Microtiter plate reader (Titertek Multiskan® MCC/340, Dynex Labsystems, Middlesex, UK) at 450 nm. Any sample with a DON value below 0.2 mg kg^{-1} was recorded as zero, whilst samples with DON values above 6 mg kg^{-1} were diluted and re-analysed. All standard curves used in this thesis had a $r^2>0.88$ (Figure 2.2).

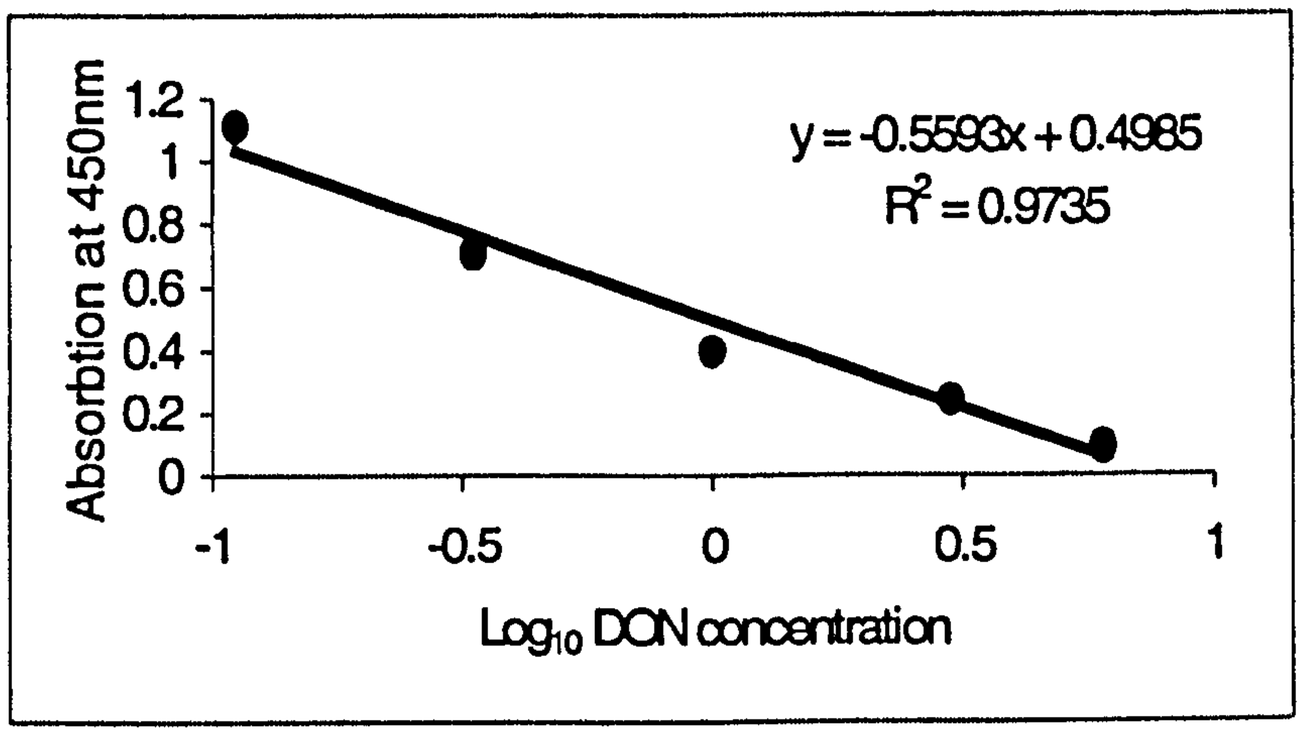


Figure 2.2 Standard curve used for DON quantification in harvested grain.

Statistical analysis

ANOVA and regression analysis was performed on all data using Genstat 5 (Release 4.1 (PC/Windows NT), Lawes Agricultural Trust, Harpenden, UK). Data were transformed to give a normal distribution where necessary.

Chapter 3

Field investigations on the effect of selected fungicides and time of application on the development of Fusarium head blight caused by *F. culmorum*, *F. graminearum* and *M. nivale* and mycotoxin accumulation in winter wheat and yield

3.1 Introduction

Effective chemical control of FHB under field conditions has generally proved inconsistent with a wealth of literature demonstrating either poor (Michail, 1989; Martin and Johnston, 1982; Gareis and Ceynova, 1994; Milus and Parsons, 1994) or successful control of the disease (Boyacioglu *et al.*, 1992; Matthies and Buchenauer, 2000; Homdork *et al.*, 2000; Cromey *et al.*, 2001). The fungicidal control of FHB has been extensively reviewed in Chapter 1.

The aim of the field study undertaken in 1998/1999 was to investigate the efficacy of selected fungicides against FHB development and DON accumulation in wheat grain. Field trials in 1999/2000 and 2000/2001 growing seasons were undertaken to determine the efficacy of fungicide spray timing on FHB development and DON accumulation in wheat grain.

Null hypothesis tested: Fungicides and their time of application do not have any effect on FHB development, mycotoxin accumulation and yield in winter wheat.

3.2 Materials and Methods

3.2.1 Field trial 1 (1998/1999)

Forty-eight plots (8 x 2 m) of the winter wheat cultivar Equinox were sown at Harper Adams University College in October 1998 into a sandy loam soil after potatoes. The crop husbandry employed and the operations carried out during the trial are listed in Appendix 2. Eight treatments were allocated to plots according to a randomised block design, using six replicate blocks. Wheat plots were inoculated at GS 65 with a conidial suspension (total concentration of 10^5 spores ml^{-1} of water) of *F. culmorum*, *F. graminearum* and *M. nivale* (Table 2.1) at a rate 33 ml m^{-2} using knapsack sprayer. All fungicides were applied when plants were at GS 59 using a gas pressurised knapsack sprayer (Safer spa, Italy) carrying four nozzles (03-F110, Lurmark, Longashton, Cambridge, UK) (Table 3.1). All plots were mist irrigated following inoculation for three weeks as described in Chapter 2.

3.2.2 Field trial 2 (1999/2000)

Thirty six plots (10 x 2 m) of the winter wheat cultivar Equinox were sown at Harper Adams University College in October 1999 into a sandy loam soil after potatoes. The crop husbandry employed and the operations carried out during the trial are listed in Appendix 3.

Nine treatments were allocated to plots according to a randomised block design, using four replicate blocks for each treatment. Plots were inoculated according to the following procedure:

Table 3.1 Fungicides and rates used in field trial 1 (1998/99)

Treatment	Fungicide	Rate (g a.i. ha^{-1})	Time of application (GS)
1	Unsprayed control		
2	metconazole	90	GS 59
3	azoxystrobin	250	GS 59
4	tebuconazole	250	GS 59
5	metconazole	45	GS 59
6	azoxystrobin	125	GS 59
7	tebuconazole	125	GS 59
8	metconazole + azoxystrobin	45 + 125	GS 59

All plots were inoculated with a 1:1:1 mixture of *F. culmorum*, *F. graminearum* and *M. nivale* at GS 65 according to the procedure detailed in Chapter 2

Five millimetre diameter mycelial discs were obtained from the edges of actively growing cultures using a sterile cork borer. Twenty discs per species (*F. culmorum*, *F. graminearum*, *M. nivale*) were placed into three five litre conical flasks containing 3 l potato dextrose broth (PDB) (Difco, Becton Dickinson and Co. Sparks, USA), which had previously been autoclaved at 121°C and 103.4 KPa for 20 minutes. The flasks were then left to stand on a laboratory bench for approximately 30 days by which time a dense growth of the pathogens were obtained.

Whilst fungal cultures were growing, 90 kg of dried shredded maize (0.5cm) was autoclaved at 121°C and 103.4 KPa for 2 hours. The amount of the shredded maize was divided into 18 large black plastic bags (5 kg per bag, 6 bags per species) and 500ml of the fungal culture were added to each bag. In order to support the initial growth of the fungi on the shredded maize, an additional 500 ml of autoclaved PDB and 500 ml of autoclaved sterile distilled water were added to each bag. The bags were kept in a room at 22°C. Bags were shaken every two days to ensure that maize was evenly coated with the fungi. After approximately one month, when plants were at growth stage 23-25, the inoculated material was evenly mixed using horizontal ribbon grain mixer (Harrison's Ltd, UK) divided after that into 36 plastic bags (2.5 kg per bag) and placed in the two middle rows of the experimental plots.

The timing and the rates of fungicides used are presented in Table 3.2. Untreated plots served as inoculated controls. Mist irrigation for this field trial started a week before GS 65 and was maintained for another three weeks after GS 65 as described in Chapter 2.

3.2.3 Field trial 3 (2000/2001)

Eighty four plots (10 x 2 m) of the winter wheat cultivar Cadenza were sown at Harper Adams University College in October 2000 into a sandy loam soil as a second wheat. The crop husbandry employed and the operations carried out during the trial are listed in Appendix 4. Twenty one treatments were allocated to plots according to a randomised block design, using four replicate blocks. Wheat plots were inoculated at GS 65 with a conidial suspension (total concentration of 10^5 spores ml^{-1} of water) of *F. culmorum*, *F. graminearum* and *M. nivale* (Table 2.1) at a rate 33 ml m^{-2} using knapsack sprayer. Fungicides were applied at half of the manufacturer's recommended dose rate. All plots were mist irrigated following inoculation for three weeks as described in Chapter 2. The timing and the rates of fungicides used are presented in Table 3.3. Untreated plots served as inoculated controls.

3.2.4 Disease Assessment

For each of the three field trials, the incidence (mean percentage of ears infected) and severity (mean percentage of spikelets infected) of FHB was determined at GS 75 and 85 by assessing 100 ears selected at random for each plot. The severity of sooty mould was assessed at GS 90 using a 1-9 scale where 1 represented healthy uninfected ears and 9 represented ears, which were entirely blackened with sooty mould.

In order to determine the effect of fungicides on the incidence of *Fusarium* spp. and *M. nivale* on wheat leaves in field trial 2 (1999/2000), ten leaves per plot were randomly taken at

Table 3.2 Fungicides, rates and time of application used in field trial 2 (1999/2000).

Treatment No.	Fungicide	Rates (g a.i. ha^{-1})	Time of application (GS)
1	Unsprayed control		
2	metconazole	90	31+39
3	metconazole	90	39+59
4	azoxystrobin	250	31+39
5	azoxystrobin	250	39+59
6	metconazole+	45 +	31+39
	azoxystrobin	125	
7	metconazole+	45 +	39+59
	azoxystrobin	125	
8	fluquinconazole	100	31+39
9	fluquinconazole	100	39+59

GS 37 (second leaf), 51and 65 (flag leaf). Leaf samples were cut into three pieces and surface sterilised in 5% sodium hypochlorite solution (0.5% available chlorine) for three minutes to eliminate surface contamination. Leaf sections were then rinsed in three changes of sterile distilled water and placed on sterile filter paper in a laminar flow cabinet to dry. Leaves were placed into Petri-dishes containing approximately 15 ml of PDA supplemented with the antibiotics

streptomycin sulphate (100 µg ml^{-1}), neomycin sulphate (50 µg ml^{-1}) and choramphenicol (50 µg ml^{-1}) at a rate of four leaf segments per plate. For identification of *M. nivale*, MBC fungicide (Bavistin, carbendazim, 50% w/w) was added to the PDA at a concentration of 10 µg ml^{-1} as described by Pettitt *et al.* (1993). Plates were incubated at 20°C+/- 2°C for 7-14 days, after which

Table 3.3 Fungicides, rates and time of application used in field trial 3 (2000/2001).

Treatment No.	Fungicide	Rates (g a.i. ha^{-1})	Time of application
1	Unsprayed control		
2	metconazole	45	5 days before inoculation (dbi)
3	tebuconazole	125	5 days before inoculation (dbi)
4	azoxystrobin	125	5 days before inoculation (dbi)
5	metconazole+ azoxystrobin	45+125	5 days before inoculation (dbi)
6	tebuconazole+ azoxystrobin	45+125	5 days before inoculation (dbi)
7	metconazole	45	2 days before inoculation (dbi)
8	tebuconazole	125	2 days before inoculation (dbi)
9	azoxystrobin	125	2 days before inoculation (dbi)
10	metconazole+ azoxystrobin	45+125	2 days before inoculation (dbi)
11	tebuconazole+ azoxystrobin	45+125	2 days before inoculation (dbi)
12	metconazole	45	2 days after inoculation (dai)
13	tebuconazole	125	2 days after inoculation (dai)
14	azoxystrobin	125	2 days after inoculation (dai)
15	metconazole+ azoxystrobin	45+125	2 days after inoculation (dai)
16	tebuconazole+ azoxystrobin	45+125	2 days after inoculation (dai)
17	metconazole	45	5 days after inoculation (dai)
18	tebuconazole	125	5 days after inoculation (dai)
19	azoxystrobin	125	5 days after inoculation (dai)
20	metconazole+ azoxystrobin	45+125	5 days after inoculation (dai)
21	tebuconazole+ azoxystrobin	45+125	5 days after inoculation (dai)

All plots were inoculated with a 1:1:1 mixture of *F. culmorum*, *F. graminearum* and *M. nivale* at GS65 according to the procedure detailed in Chapter 2

time the isolates were identified according to Booth (1971).

Isolates which could not be identified on PDA were sub-cultured onto low nutrient agar (Nierenberg, 1981) (Appendix 1) to allow microscopic examination of spore morphology to aid identification.

All plots were harvested when ripe (GS 92) and the moisture content and total grain yield recorded. Two-kilogram samples were then taken from each plot and specific weight and thousand grain weight determined. From each two-kilogram grain sample, a sub-sample of approximately 230 grams was taken using ripple grain divider (Novaliter Laboratories, UK) and trichothecene-producing *Fusarium* spp. and *M. nivale* were quantified as described in Chapter 2. In field trial 1 (1998/1999) grain contamination was also examined by plating 200 grain per plot on PDA. Grain samples were surface sterilised in 5% sodium hypochlorite solution (0.5% available chlorine) for three minutes to eliminate surface contamination. Grain were then rinsed in three changes of sterile distilled water and placed on sterile filter paper in a laminar flow cabinet to dry. Dry grain were placed into Petri-dishes containing approximately 15 ml of PDA supplemented with the antibiotics streptomycin sulphate (100 $\mu g\ ml^{-1}$), neomycin sulphate (50 $\mu g\ ml^{-1}$) and choramphenicol (50 $\mu g\ ml^{-1}$) at rate five grain per plate. For identification of *M. nivale*, MBC fungicide (Bavistin, carbendazim, 50% w/w) was added to the PDA at a concentration of 10 $\mu g\ ml^{-1}$ as described by Pettitt *et al.* (1993). Plates were incubated at 20°C+/- 2°C for 7-14 days, after which time the isolates were identified according to Booth (1971). Isolates which could not be identified from PDA were sub-cultured onto low nutrient agar (Nierenberg, 1981) (Appendix 1) to allow microscopic examination of spore morphology to aid identification.

DON concentration in harvested grain was quantified using Ridascreen®DON Fast immunoassay (R-Biopharm Rhône Ltd, Glasgow, UK) according to the manufacturer's instruction (see Chapter 2).

3.3 Results

3.3.1 Field trial 1 (1998/1999).

The effect of fungicides on the incidence and severity of FHB in field trial 1 (1998/1999) are presented in Table 3.4 and Appendix 5, respectively. Analysis of variance revealed significant differences between the treatments for both assessment dates (21 days (GS 75) and 28 days (GS 85) post inoculation). For example, at GS 85 a mixture of metconazole and azoxystrobin provided 18% reduction of disease incidence when compared to the control treatment. With the exception of tebuconazole applied at half rate, which was not significantly different to the control, all other treatments reduced disease incidence by 2-4% over the untreated control for the same assessment date. More noticeable effects of fungicide treatments were observed against the severity of FHB. For example, the mixture of metconazole and azoxystrobin reduced the severity of FHB symptoms by 62% at GS 75 and by 63% at GS 85 when compared to the control. Tebuconazole and metconazole applied at full rate reduced disease severity by 45% and 39%, respectively, when compared to the control. The effect of half rate of the same fungicides against FHB development was not significantly different that those applied at full rate ($P>0.05$). Azoxystrobin applied on its own was the least effective treatment at controlling disease severity.

Competitive PCR was employed to quantify trichothecene-producing *Fusarium* species in harvested grain (see Chapter 2). Table 3.5 shows the $\log_{10}$ *Tri*5 DNA mean concentration obtained for each fungicide treatment tested. Metconazole and tebuconazole applied at full rate reduced *Tri*5 DNA in grain by 84% and 75%, respectively, when compared to that recorded in grain from the untreated control. Azoxystrobin at both concentrations and the metconazole-azoxystrobin mixture had no significant effect on the *Tri*5 DNA concentration in harvested grain.

The most effective treatments at reducing DON concentration in grain were metconazole and tebuconazole applied at full rate (Table 3.5). Both fungicides reduced DON in grain by 83%, whilst azoxystrobin had no significant effect on the DON quantity in grain whether applied at full or half dose rate.

Table 3.4 The effect of fungicides applied at GS 59 to plots of winter wheat (cv Equinox) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the severity of FHB recorded 21 and 28 days post-inoculation in field trial 1 (1998/1999). Numbers in parentheses are back-transformed means

Treatment	Fungicides	Rates (g.a.i. ha^{-1})	Arcsine % spikelets infected GS 75	Arcsine % spikelets infected GS 85
1	Unsprayed control		28.40 (22.62)	33.37 (30.25)
2	metconazole	90	24.39 (17.05)	25.47 (18.49)
3	azoxystrobin	250	22.19 (14.26)	29.67 (24.50)
4	tebuconazole	250	22.97 (15.22)	24.22 (16.82)
5	metconazole	45	24.31 (16.94)	27.03 (20.65)
6	azoxystrobin	125	21.67 (13.63)	27.49 (21.30)
7	tebuconazole	125	22.41 (14.53)	26.33 (19.67)
8	metconazole	45 +	17.09 (08.63)	19.60 (11.25)
	azoxystrobin	125		
LSD (5%)			2.19 (P<0.001) CV= 8.2 %	2.45 P<0.001) CV= 7.9 %

Table 3.5 The effect of fungicides applied at GS 59 to plots of winter wheat (cv Equinox) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the quantity of *Tri5* DNA and DON concentration in grain in field trial 1 (1998/1999). Numbers in parentheses are back-transformed means

Treatment	Fungicide	Rates (g. a.i. ha^{-1})	Log_{10} *Tri5* DNA (pg ng^{-1} of total DNA)	DON (mg kg^{-1})
1	Unsprayed control		0.879 (7.57)	11.29
2	metconazole	90	0.092 (1.23)	2.00
3	azoxystrobin	250	1.007 (10.1)	12.21
4	tebuconazole	250	0.281 (1.91)	1.89
5	metconazole	45	0.546 (3.52)	5.58
6	azoxystrobin	125	0.794 (6.22)	10.37
7	tebuconazole	125	0.399 (2.51)	3.84
8	metconazole+ azoxystrobin	45 + 125	0.706 (5.08)	6.71
LSD (5%)			0.205 (P<0.001) CV = 29.9 %	2.01 (P<0.001) CV = 25.5 %

The incidence of *Fusarium* species and *M. nivale* in the harvested grain are shown in Appendix 7. None of the fungicide treatments had a significant effect ($P>0.05$) on the incidence of *F. culmorum* and *F. graminearum* in harvested grain. The incidence of *M. nivale* in grain was significantly affected by azoxystrobin applied at both rates (81% and 74% reduction, respectively) and by the metconazole-azoxystrobin mixture (68% reduction). Metconazole and tebuconazole applied at both rates significantly increased the incidence of *M. nivale* by 35–50% when compared to that recorded for the untreated control.

The effect of fungicides on the severity of sooty moulds in presented in Appendix 5. Azoxystrobin applied at full rate provided the most significant reduction of sooty moulds followed by metconazole-azoxystrobin mixture.

All fungicide treatments significantly increased grain yield ($P<0.001$) (Appendix 11). The most effective treatment was the metconazole-azoxystrobin mixture, which resulted in a yield increase of 27%. The other treatments also significantly increased grain yield between 13 and 21% compared to control treatment but there was no significant difference between them. Metconazole and tebuconazole applied at both rates had a significant effect on 1000 grain weight and the specific grain weight, whilst azoxystrobin applied at full rate had no effect on 1000 grain weight and no effect on the specific grain weight for both rates of application (Appendix 11).

3.3.2 Field trial 2 (1999/2000).

In field trial 2 (1999/2000), due to the favourable conditions for disease development, the incidence of FHB at GS 85 was 100%. The effect of the fungicide programmes on FHB development can be seen in Table 3.6. At GS 75, assessment of FHB severity indicated that fungicides applied at GS 31+39 and at GS 39+59 reduced significantly disease by 26% and 42%, respectively, over the untreated control plots. This could suggest that early season treatments could have a beneficial effect against FHB. However, with the significant disease progression, which developed throughout the season, such a treatment effect, was not evident following assessment of disease severity at GS 85. Factorial ANOVA revealed that fungicides applied at GS 39+59 reduced disease severity by

Table 3.6 The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize infected with *F. culmorum*, *F. graminearum* and *M. nivale* at GS 23-25 on the severity of FHB recorded 21 and 28 days post-flowering in field trial 2 (1999/2000). Numbers in parentheses are back-transformed means.

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of application	Arcsine % spikelets infected GS 75	Arcsine % spikelets infected GS 85
1	Unsprayed control			23.63 (16.06)	41.70 (44.25)
2	metconazole	90	31+39	19.49 (11.13)	39.30 (40.11)
3	metconazole	90	39+59	17.78 (09.32)	34.05 (31.13)
4	azoxystrobin	250	31+39	21.06 (12.91)	39.49 (40.44)
5	azoxystrobin	250	39+59	17.21 (08.75)	32.69 (29.17)
6	metconazole+ azoxystrobin	45 + 125	31+39	19.65 (11.30)	37.08 (36.35)
7	metconazole+ azoxystrobin	45 + 125	39+59	13.47 (5.42)	28.46 (22.70)
8	fluquinconazole	100	31+39	20.81 (12.62)	39.01 (39.62)
9	fluquinconazole	100	39+59	22.85 (15.07)	40.40 (42.00)
LSD (5%)	Fungicide			1.89 (P<0.001) CV = 7.7 %	2.75 (P<0.001) CV = 5.9 %
LSD (5%)	Time			1.73 (P<0.001) CV = 7.7 %	2.51 (P<0.001) CV = 5.9 %
LSD (5%)	Fungicide*time			2.19 (P<0.001) CV = 7.7 %	3.17 (P<0.001) CV = 5.9 %

whilst treatments applied early in the season (GS 31+39) had no effect on FHB severity. The interaction between fungicides and the time of their application was significant and indicated that although a mixture of metconazole and azoxystrobin significantly reduced disease severity in both fungicide programmes, this treatment provided significantly better control of FHB when applied at GS 39+59 reducing disease by 49% in comparison with the control, whilst earlier application of this mixture (GS 31+39) reduced FHB by only 18%. Azoxystrobin or metconazole applied alone at GS 39+59 also provided significant disease reduction by 34 and 29%, respectively. However, there was no significant difference between these two treatments, which is probably due to the high natural background of *M. nivale* present on the wheat ears.

The assessment of sooty moulds (Appendix 10) showed that fungicides applied at GS 39+59 were more effective at reducing the severity of sooty mould than those applied earlier in the season and the mixture of metconazole and azoxystrobin applied at GS 39+59 reduced the disease by 65% in comparison with untreated control.

Similar results were observed for the quantification of trichothecene producing *Fusarium* species (*Tri*5 DNA) in wheat grain (Table 3.7). Applications of either metconazole or metconazole+ azoxystrobin at GS 39+59 were shown to be the most effective treatments. Quantification of *M. nivale* in grain, a non-mycotoxin producing pathogen which is one of a complex of species causing FHB, showed that both azoxystrobin and metconazole+azoxystrobin mixture applied at GS 39+59, reduced significantly ($P<0.01$) the amount of this pathogen's DNA in harvested grain by 76% and 73%, respectively, when compared to untreated control. Visual assessment of grain samples (Table 3.8) revealed that metconazole applied at GS 39+59 was the only effective treatment capable of reducing the incidence of *Fusarium* damaged kernels (FDK) (31%). Analysis of DON concentration in grain (Table 3.8) showed that overall, metconazole whether applied at GS31+39 or GS 39+59 decreased DON concentration when compared with the other fungicides. The time of fungicide application did not have any effect on the fungicide performance. Although the interaction between fungicides and time of application was not significant, when metconazole

Table 3.7 The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize infected with *F. culmorum*, *F. graminearum* and *M. nivale* at GS 23-25 on the quantity of *Tri*5 DNA and *M. nivale* DNA in harvested grain in field trial 2 (1999/2000). Numbers in parentheses are back-transformed means

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of application	Log_{10} *Tri*5 DNA (pg ng^{-1} of total DNA)	Log_{10} *M.nivale* DNA (pg ng^{-1} of total DNA)
1	Unsprayed control			0.718 (5.22)	1.389 (24.49)
2	metconazole	90	31+39	1.049 (11.19)	1.160 (14.45)
3	metconazole	90	39+59	0.644 (04.41)	1.236 (17.21)
4	azoxystrobin	250	31+39	1.136 (13.67)	1.071 (11.77)
5	azoxystrobin	250	39+59	1.109 (12.85)	0.761 (05.76)
6	metconazole+ azoxystrobin	45 + 125	31+39	0.850 (07.08)	1.271 (18.66)
7	metconazole+ azoxystrobin	45 + 125	39+59	0.644 (04.41)	0.816 (06.54)
8	fluquinconazole	100	31+39	1.123 (13.27)	1.229 (16.94)
9	fluquinconazole	100	39+59	1.087 (12.21)	1.005 (10.11)
LSD (5%)	Fungicide			0.165 (P<0.001) CV = 14.1 %	0.209 (P<0.01) CV = 15.0 %
LSD (5%)	Time			0.150 (P<0.001) CV = 14.1 %	0.191 (P<0.001) CV = 15.0 %
LSD (5%)	Fungicide*time			0.190 (P<0.001) CV = 14.1 %	0.241 (P<0.01) CV = 15.0 %

Table 3.8 The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize infected with *F. culmorum, F. graminearum* and *M. nivale* at GS 23-25 on the concentration of DON in grain and Fusarium Damaged Kernels (FDK) in field trial 2 (1999/2000).

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of Application	DON mg kg^{-1}	% FDK
1	Unsprayed control			40.4	37.50
2	metconazole	90	31+39	36.5	39.00
3	metconazole	90	39+59	15.6	25.87
4	azoxystrobin	250	31+39	40.6	37.63
5	azoxystrobin	250	39+59	44.1	39.75
6	metconazole+ azoxystrobin	45 + 125	31+39	33.6	36.13
7	metconazole+ azoxystrobin	45 + 125	39+59	41.4	34.48
8	fluquinconazole	100	31+39	32.4	37.00
9	fluquinconazole	100	39+59	30.7	39.88
LSD (5%)	Fungicide			13.57 (P<0.01) CV = 30.6 %	6.65 (P>0.05) CV = 14.5 %
LSD (5%)	Time			12.38 (P>0.05) CV = 30.6 %	6.07 (P>0.05) CV = 14.5 %
LSD (5%)	Fungicide*time			15.66 (P>0.05) CV = 30.6%	7.68 (P<0.01) CV = 14.5 %

was applied at GS 39+59, DON concentration in grain was reduced by 62% when compared to untreated control.

The effect of fungicide programmes on the incidence of *Fusarium* species and *M. nivale* isolated from leaves taken at GS 37, GS 51 and GS 65 are shown in Appendices 8, 9 and 10, respectively. Factorial analysis of the data for the three assessment dates showed no significant effect of fungicide treatments and the time of their application ($P>0.05$) on the incidence of FHB pathogens on wheat leaves. It could be suggested that this insignificant effect was a result of the high amount of inoculum provided at the end of tillering and that although fungicide treatments may have provided a significant effect on the incidence of FHB pathogens on the wheat leaves immediately after their application, within two-three days, the wheat leaves may have been reinfected by fungal propagules dispersed during subsequent rainfall events.

The yield data obtained from field trial 2 (1999/2000) is presented in Appendix 12. The fungicide programmes provided a significant increase in the yield of wheat ($P=0.001$). However, when fungicides were applied at GS 39+59, yield increased by 19% compared with control treatment, whilst the earlier fungicide application increased yield by 11%. The interaction between fungicides and time application was significant ($P=0.001$) and revealed that when a metconazole-azoxystrobin mixture or when metconazole was applied alone at GS 39+59, yield increased by 32% and 25% respectively. Thousand grain weight (TGW) was significantly affected by the time of fungicide application ($P=0.001$). For example, fungicide applications at GS 39+59 increased TGW by 27%. None of fungicide treatments tested had a significant effect on specific grain weight ($P>0.05$).

3.3.3 Field trial 3 (2000/2001).

Factorial ANOVA for field trial 3 (2000/2001) revealed that all fungicide treatments significantly reduced ($P<0.001$) disease incidence when plots were assessed at GS 75 (Appendix 6). Fungicides applied 2 days before inoculation and 2 days after inoculation provided the most significant reduction of disease incidence. There was no significant interaction determined between fungicide and timing of application ($P>0.05$). As the disease progressed, the effect of fungicide timing on the

Table 3.9 The effect of fungicides applied at different timing to plots of winter wheat (cv Cadenza) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the severity of FHB in field trial 3 (2000/2001). Numbers in parentheses are back-transformed means

Treatment No.	Fungicide	Rates (g a. i. ha^{-1})	Time of application	Arcsine % spikelets infected GS 75	Arcsine % spikelets infected GS 85
1	Unsprayed control			11.02 (3.65)	17.95 (9.49)
2	metconazole	45	5 dbi*	6.87 (1.43)	13.16 (5.18)
3	tebuconazole	125	5 dbi	6.84 (1.41)	12.73 (4.85)
4	azoxystrobin	125	5 dbi	8.78 (2.32)	15.29 (6.95)
5	metconazole+ azoxystrobin	45+125	5 dbi	6.58 (1.31)	12.01 (4.32)
6	tebuconazole+ azoxystrobin	45+125	5 dbi	5.90 (1.05)	12.36 (4.58)
7	metconazole	45	2 dbi	5.43 (0.89)	12.01 (4.32)
8	tebuconazole	125	2 dbi	6.83 (1.41)	11.72 (4.12)
9	azoxystrobin	125	2 dbi	9.13 (2.51)	14.67 (6.41)
10	metconazole+ azoxystrobin	45+125	2 dbi	5.25 (0.83)	10.70 (3.44)
11	tebuconazole+ azoxystrobin	45+125	2 dbi	5.10 (0.79)	10.72 (3.45)
12	metconazole	45	2 dai**	4.56 (0.63)	11.53 (4.00)
13	tebuconazole	125	2 dai	6.94 (1.45)	11.63 (4.06)
14	azoxystrobin	125	2 dai	8.17 (2.01)	14.54 (6.31)
15	metconazole+ azoxystrobin	45+125	2 dai	5.15 (0.80)	10.07 (3.05)
16	tebuconazole+ azoxystrobin	45+125	2 dai	4.85 (0.71)	10.60 (3.38)
17	metconazole	45	5 dai	7.01 (1.48)	14.50 (6.26)
18	tebuconazole	125	5 dai	6.53 (1.29)	11.92 (4.26)
19	azoxystrobin	125	5 dai	8.62 (2.46)	14.50 (6.27)
20	metconazole+ azoxystrobin	45+125	5 dai	6.36 (1.22)	11.85 (4.21)
21	tebuconazole+ azoxystrobin	45+125	5 dai	5.53 (0.92)	12.06 (4.36)
LSD (5%) Fungicide				1.37 (P<0.001) CV=18.2 %	1.03 (P<0.001) CV=7.3%
LSD (5%) Time				1.34 (P<0.001) CV=18.2 %	1.01 (P<0.001) CV=7.3%
LSD (5%) Fungicide*time				1.73 (P>0.05) CV=18.2 %	1.30 (P>0.05) CV=7.3%

* days before inoculation;
** days after inoculation

incidence of FHB became less noticeable and at the second assessment date (GS 85) the timing of fungicides had no significant effect on disease incidence ($P>0.05$).

For both assessment dates, all fungicides had a significant effect on FHB severity (Table 3.9). For example, overall at GS 85, all triazole fungicides and their mixtures reduced visual symptoms by up to 61% compared the untreated plots. The effect of fungicides also varied depending on their time of application ($P<0.001$). Although there was a significant reduction of disease severity between the four fungicide timings over the control, the second visual assessment at GS 85 indicated that treatments applied 2 days pre-inoculation and 2 days post-inoculation were the most effective at reducing FHB symptoms (55-57%). The severity of sooty moulds on the wheat ears was not significantly affected either by the fungicides used in this study, or by their time of application (Appendix 6).

Quantification of *Tri*5 DNA indicated that those treatments which contained triazole fungicides resulted in a significant reduction ($P<0.001$) of trichothecene-producing *Fusarium* in grain over the control (Table 3.10). Conversely, azoxystrobin had no significant effect on the amount of *Tri*5 DNA. Factorial ANOVA revealed a significant interaction between fungicides and their time of application ($P<0.05$). Metconazole, applied 2 days pre- and 2 days post-inoculation were the most effective treatments and reduced *Tri*5 DNA in grain by 57% and 60%, respectively. Tebuconazole significantly reduced *Tri*5 DNA over the control treatment but was not affected by the time of application. Mixtures of the two triazole fungicides with azoxystrobin also provided significant reduction of trichothecene-producing species and were most effective when applied either 2 days pre- or 2 days post-inoculation.

The results for the quantity of *M. nivale* DNA in harvested grain are presented in Table 3.10. In general, ANOVA indicated that those treatments which contained azoxystrobin significantly reduced *M. nivale* content in grain by between 43 and 52%. Treatments with either metconazole or tebuconazole had no effect on *M. nivale* colonisation of grain. Fungicides applied 5 pre-inoculation had no significant effect ($P>0.05$) on *M. nivale* DNA concentration in grain when compared to the

control treatment. No significant interaction between fungicide treatments and their time of application was observed ($P>0.05$).

The effect of fungicides and the time of their application on DON concentration in harvested wheat grain is shown in Table 3.10. The overall effect of fungicides indicated that there was a significant reduction in DON in grain when compared to the control treatment ($P<0.001$). Furthermore, the time of fungicide application also influenced fungicide performance against DON. Factorial analysis revealed that treatments applied either 2 days pre-, 2 days post- or 5 days post-inoculation had the most significant effect on DON concentration over the untreated control. Metconazole applied 2 days post-inoculation proved the most effective treatment reducing DON concentration by 84%. Although tebuconazole showed a reduction in DON between 39 and 54%, the effect of this fungicide on DON concentration was unaffected by the different times of application. Azoxystrobin showed some reduction of DON in grain when applied 5 days pre- and 5 days post-inoculation.

The fungicides in this trial had variable effects on yield and its parameters. Metconazole, metconazole+azoxystrobin, and tebuconazole+azoxystrobin all significantly increased yield by 3% - 5%. The effect of fungicide timing had no effect on grain yield. None of the fungicide treatments had any effect on either 1000- grain weight or specific grain weight.

3.4 Relationships between disease incidence, severity, deoxynivalenol, *Tri*5 DNA, and yield.

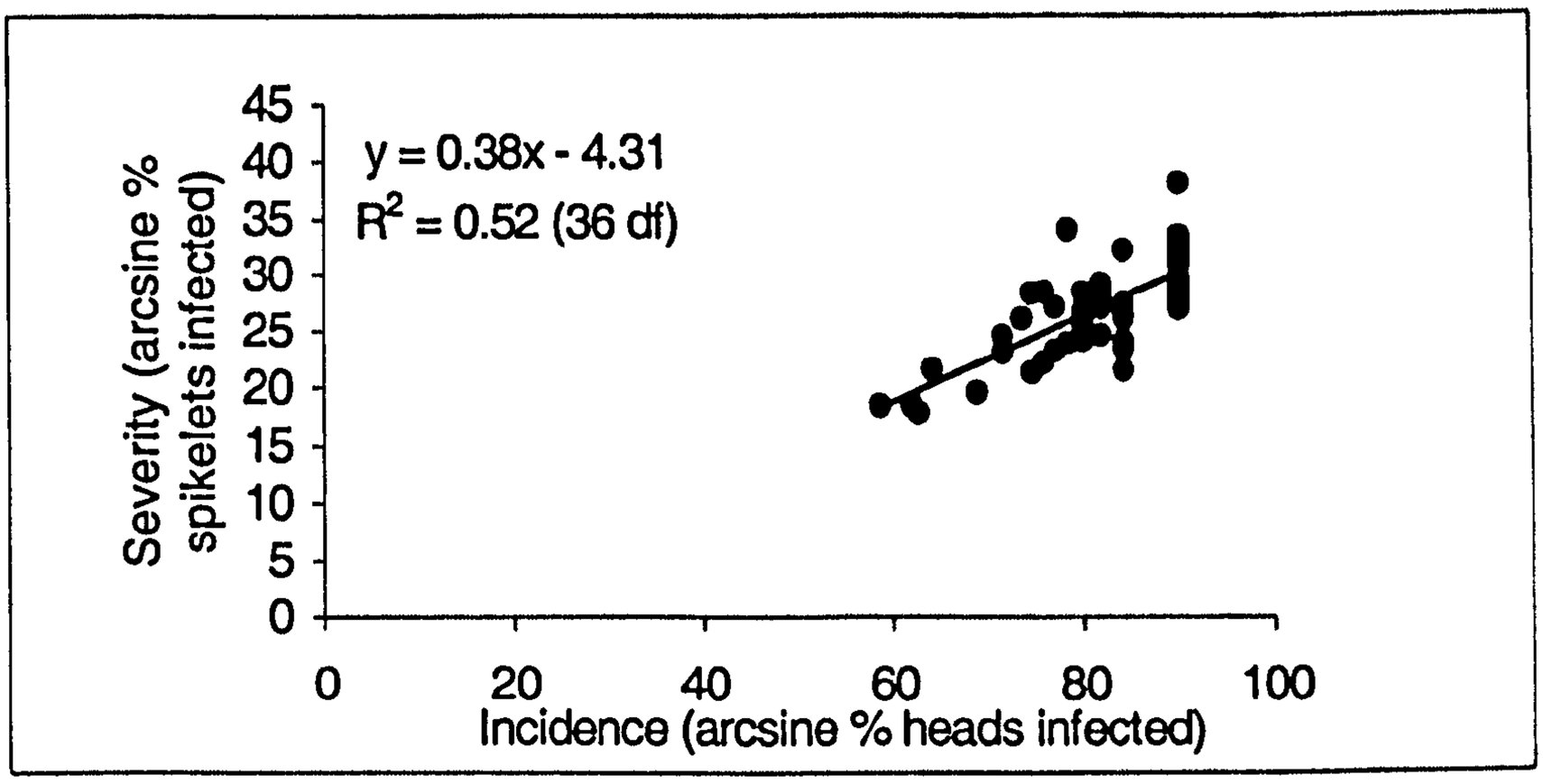
Regression analysis of the data from field trial 1 (1998/1999) showed no relationship ($P>0.05$) between disease severity and *Tri*5 DNA, DON concentration in grain and grain yield. However, a significant and strong relationship ($P<0.001$) was observed between FHB incidence and severity (Figure 3.1a) and between the $\log_{10}$ *Tri*5 DNA quantity and DON concentration (Figure 3.2a). Such relationships were also observed in field trial 3 (2000/01) (Figure 3.1b; 3.2b). Regression analysis revealed no significant relationship between any of the variables recorded during field trial 2 (1999/2000).

Table 3.10 The effect of fungicides applied at different timing to plots of winter wheat (cv Cadenza) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the quantity of *Tri5* DNA, *M.nivale* DNA and DON from grain samples in field trial 3 (2000/2001). Numbers in parentheses are back-transformed means

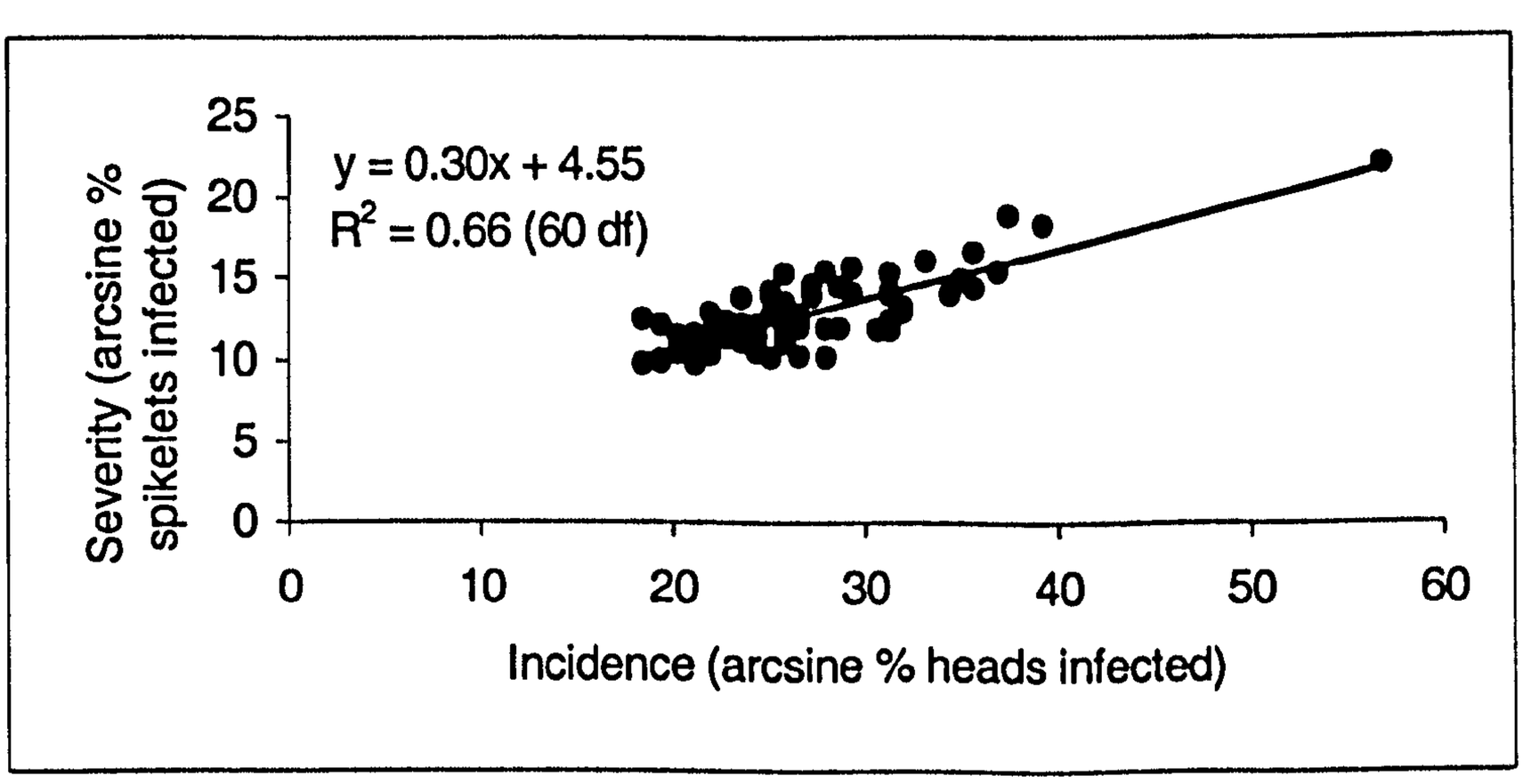
Treatment No.	Fungicide	Rates (g a. i. ha^{-1})	Time of application	Log_{10} *Tri5* DNA (pg ng^{-1} of total DNA)	Log_{10} *M.nivale* DNA (pg ng^{-1} of total DNA)	DON mg kg^{-1}
1	Unsprayed control			0.572 (3.73)	0.634 (4.31)	2.09
2	metconazole	45	5 dbi*	0.435 (2.72)	0.617 (4.14)	1.32
3	tebuconazole	125	5 dbi	0.394 (2.47)	0.842 (6.95)	1.23
4	azoxystrobin	125	5 dbi	0.428 (2.67)	0.543 (2.68)	1.00
5	metconazole+ azoxystrobin	45+ 125	5 dbi	0.497 (3.14)	0.521 (3.31)	1.44
6	tebuconazole+ azoxystrobin	45+ 125	5 dbi	0.452 (2.83)	0.617 (4.13)	1.31
7	metconazole	45	2 dbi	0.205 (1.60)	0.616 (4.13)	0.66
8	tebuconazole	125	2 dbi	0.442 (2.64)	0.508 (3.22)	1.02
9	azoxystrobin	125	2 dbi	0.580 (3.80)	0.473 (2.97)	1.62
10	metconazole+ azoxystrobin	45+ 125	2 dbi	0.335 (2.26)	0.362 (2.30)	0.76
11	tebuconazole+ azoxystrobin	45+ 125	2 dbi	0.379 (2.39)	0.305 (2.01)	0.66
12	metconazole	45	2 dai**	0.172 (1.48)	0.643 (4.39)	0.32
13	tebuconazole	125	2 dai	0.419 (2.62)	0.529 (3.38)	0.95
14	azoxystrobin	125	2 dai	0.722 (5.40	0.427 (2.67)	1.82
15	metconazole+ azoxystrobin	45+ 125	2 dai	0.405 (2.54)	0.230 (1.69)	0.68
16	tebuconazole+ azoxystrobin	45+ 125	2 dai	0.288 (1.94)	0.302 (2.00)	0.58
17	metconazole	45	5 dai	0.382 (2.40)	0.456 (2.85)	0.65
18	tebuconazole	125	5 dai	0.409 (2.56)	0.486 (3.06)	1.03
19	azoxystrobin	125	5 dai	0.643 (4.39)	0.181 (1.51)	1.40
20	metconazole+ azoxystrobin	45+ 125	5 dai	0.492 (3.10)	0.300 (1.99)	1.18
21	tebuconazole+ azoxystrobin	45+ 125	5 dai	0.342 (2.19)	0.321 (2.09)	0.78
LSD (5%) Fungicide				0.128 (P<0.001) CV= 26.8%	0.199 (P<0.001) CV=37.6%	0.45 (P<0.001) CV=37.9%
LSD (5%) Time				0.125 (P>0.05) CV= 26.8%	0.195 (P<0.001) CV=37.6%	0.44 (P<0.05) CV=37.9%
LSD (5%) Fungicide*time				0.162 P<0.05) CV= 26.8%	0.252 (P>0.05) CV=37.6%	0.57 (P<0.05) CV=37.9%

* days before inoculation

** days after inoculation

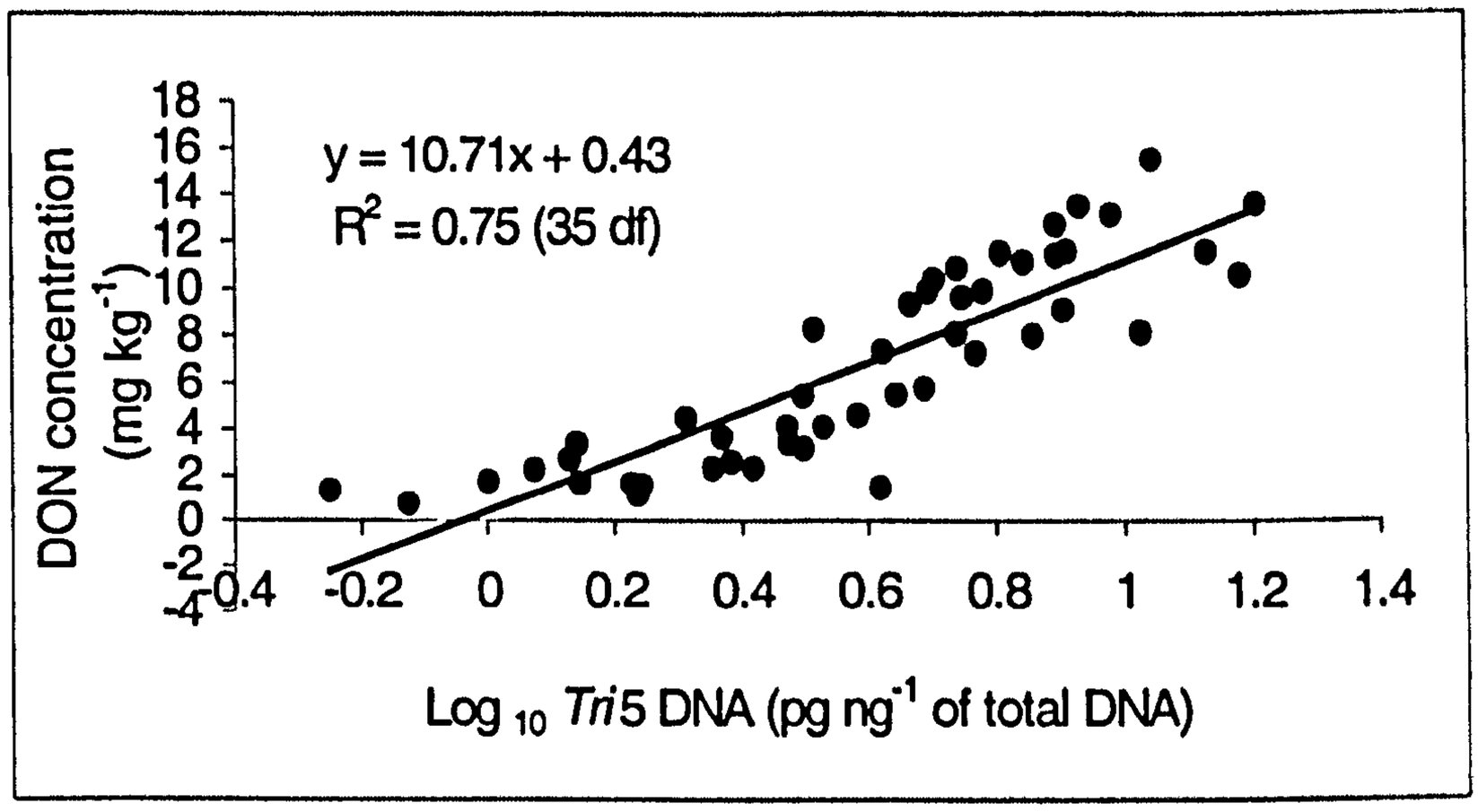


a

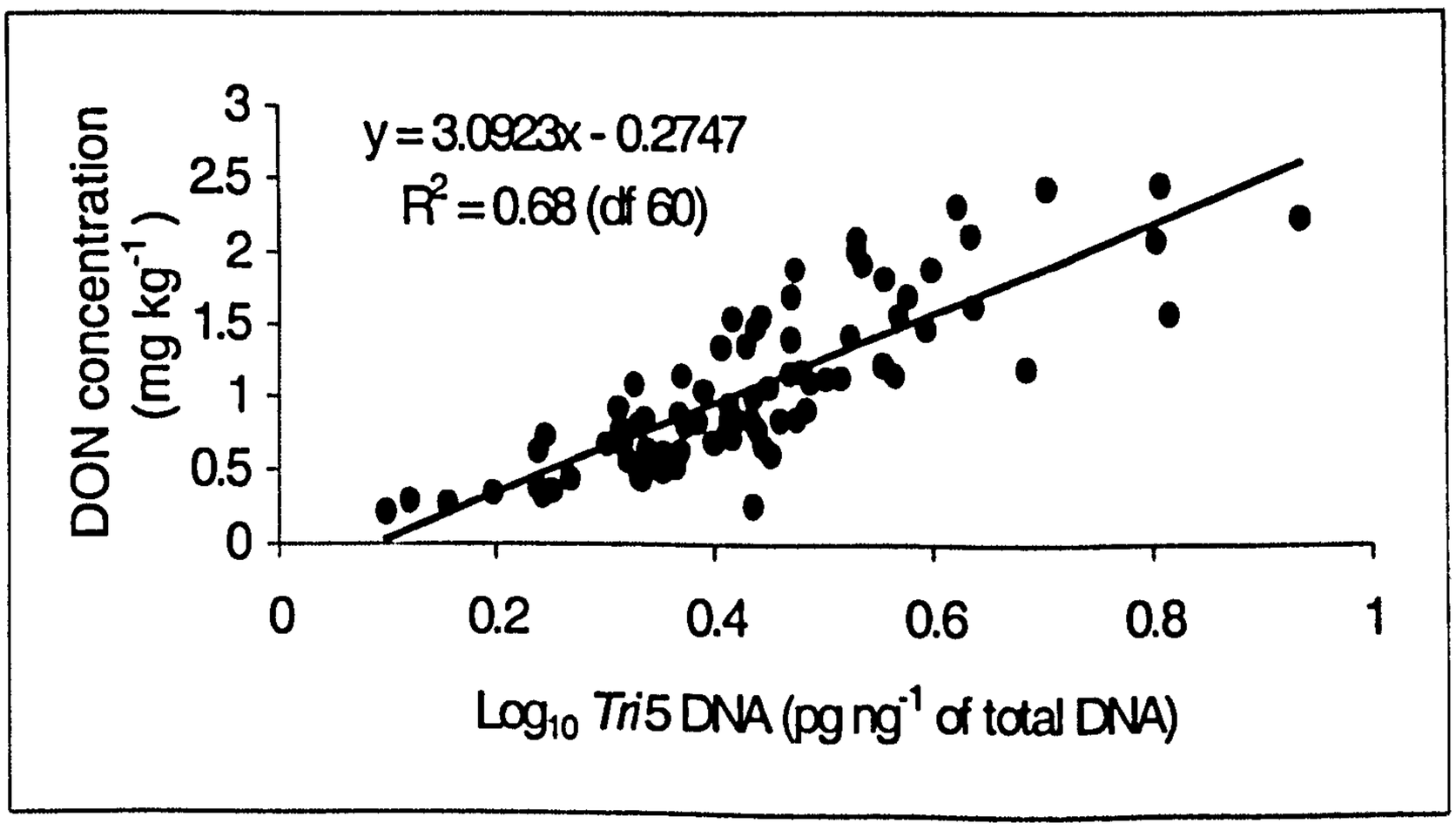


b

Figure 3.1 The relationship between incidence (arcsine % heads infected) and severity (arcsine % spikelets infected) of FHB in winter wheat assessed at GS 85 (a) in field trial 1 (1998/99) and (b) in field trial 3 (2000/02)



a



b

Figure 3.2 Relationship between quantity of *Tri*5 DNA and DON concentration in grain of winter wheat in (a) field trial 1 (1998/99) and (b) field trial 3 (2000/01)

3.5 Discussion

Fungicide efficacy against FHB has in the past proved to be inconsistent. For example, Milus and Parsons (1994), after applying tebuconazole to a field trial of winter wheat artificially inoculated with *Fusarium graminearum*, failed to record any significant reduction in disease severity. In contrast, Suty and Mauler-Machnik (1996) showed that applications of tebuconazole at either GS 55 or GS 65 led to a significant decrease in FHB caused by *F. graminearum*. The results from field trial 1 (1998/1999) demonstrated that a mixture of metconazole+azoxystrobin applied at GS 59 was effective at reducing FHB severity by 63% and that applications of tebuconazole or metconazole were also effective at reducing disease severity (45% and 39% control, respectively). Azoxystrobin proved the least effective at controlling FHB. These observations may be explained by the fact that wheat plots were artificially inoculated with a mixture of the FHB pathogens *F. culmorum, F. graminearum* and *M. nivale* (see Table1.2). In field trials carried out by Simpson *et al.* (2001), applications of tebuconazole where shown to reduce significantly ($P<0.001$) the amount of *Fusarium* spp. in harvested grain but had no significant effect on levels of *M. nivale* ($P>0.2$). In contrast, fungicide treatments containing azoxystrobin effectively controlled *M. nivale* ($P<0.01$) but did not significantly reduce levels of *Fusarium* species ($P>0.2$).

Results from this study are also in agreement with the work of Dardis and Walsh (2000) who found that applications of either metconazole, tebuconazole or a mixture of metconazole with azoxystrobin to plots of spring wheat inoculated with *F. culmorum*, resulted in significantly less disease ($P<0.01$) in comparison with untreated plots. Jones (2000) also reported that applications of tebuconazole and propiconazole against FHB (*F. graminearum*) in barley significantly reduced FHB severity by 39% and 31%, whilst azoxystrobin was the least effective treatment.

A PCR-based assay using primers derived from the trichodiene synthase gene (*Tri*5), an enzyme which catalyses the initial reaction in the biosynthetic pathway of all trichothecene mycotoxins revealed that metconazole and tebuconazole applied at full rate provided effective control of trichothecene-producing *Fusarium* and reduced *Tri*5 DNA in harvested grain by 84 and 75%, respectively. Metconazole and tebuconazole were also shown to significantly reduce the

concentration of DON in harvested grain by 82% and 83%, respectively. Azoxystrobin applied at both rates had no significant effects on either *Tri*5 DNA quantity in grain or DON concentration (P>0.05). These results are contrary to data obtained from several *in vitro* and *in vivo* studies. For example, *in vitro* work by Matthies *et al.* (1999) showed that tebuconazole at three sub-lethal concentrations (1, 0.5 and 0.1 μg ml^{-1} of PDB) significantly (P<0.05) reduced mycelial growth of *F. graminearum* on potato dextrose broth and that 3-ADON (3-acetyldeoxynivalenol) concentration at 0.1 μg ml^{-1} of fungicide increased four times in comparison with control. In addition, thiabendazole reduced fungal growth at 1, 2, 2.5 μg ml^{-1}, but at 1 μg ml^{-1} 3-ADON concentration considerably increased compared to the fungicide-free control.

Studies on naturally infected field trials in the Atlantic Provinces in Canada by Martin and Johnston (1982) showed that propiconazole at a rate of 250g a.i. ha^{-1} applied at growth stages GS 51 and GS 73 provided good control of FHB but did not affect DON concentration in harvested grain. Milus and Parsons (1994) studied the effect of benomyl, chlorothalonil, fenbuconazole, flusilazole, myclobutanil, potassium bicarbonate, propiconazole, tebuconazole, thiabendazole and triadimefon plus mancozeb against FHB severity, DON contamination and yield of winter wheat. During two years of field studies, these authors did not observed any significant effect of these fungicides on either FHB or DON levels in harvested grain. Field work of Jennings *et al.* (2000) attributed application of azoxystrobin to significant increases in DON production in comparison to control treatment. They also (Jennings *et al.*, 2000) suggested that applications of azoxystrobin reduced *M. nivale* on wheat ears and altered the proportion of trichothecene producing *Fusarium* within the complex which in turn resulted in an increase in mycotoxin production. In contrast with these findings this study failed to observe any direct adverse effects of azoxystrobin on DON production. Indeed, the strong relationship between *Tri*5 DNA and DON in grain (Figure 3.2) indicated that DON concentration in grain increased linearly as the amount of trichothecene producing *Fusarium* in grain increased. If a treatment had resulted in a cluster of points above or below the regression line, this would have indicated that this treatment increased or decreased, respectively, the amount of DON produced per *Tri*5 copy present. The lack of any obvious cluster

indicates that in the field, these fungicides did not influence directly the DON concentration within grain other than by altering the amount of trichothecene producing *Fusarium* present.

Plating out of grain onto PDA in this trial showed no effect ($P>0.05$) of the fungicides on the incidence of *Fusarium* spp. in grain, whilst the presence of *M. nivale* was significantly ($P<0.001$) reduced by treatments containing azoxystrobin. The lack of effect of fungicides on the incidence of *Fusarium* spp. in grain is in agreement with the findings of Michail (1989) who observed that seed colonisation of 32 samples of 16 wheat varieties from crops sprayed with either triadimefon, captfol+triadimefon, fenpropimorph, carbendazim, propiconazole, captafol+pyrazofos or prochloraz at different growth stages between GS 32 and GS 50 (Zadoks *et al.*, 1974) did not differ significantly in the incidence of *Fusarium* spp. present. One possible reason could be that the fungal colonies growing from individual grains may originate from either one single spore or from heavily colonised grain when using the plate count technique. A more accurate way to evaluate fungicide performance would be to quantify the amount of fungus present in grain using competitive Polimerase Chain Reaction (PCR) as has been demonstrated by Glynn *et al.* (2000).

The timing of fungicide application appears to be an important factor in effectively suppressing FHB with fungicides. An attempt to investigate the effect of fungicide application early in the growing season was proposed by Huthceon and Jordan (1992). During glasshouse studies, these workers showed that an application of tebuconazole+triadimenol at GS 49 or GS 69 to wheat plants inoculated with a conidial suspension of *Fusarium* spp. at the seedling stage, reduced severity of FHB by 48% and 71%, respectively when compared to the control treatment. When tebuconazole was applied at GS 31, GS 39, GS 55 or GS 39+55, a significant reduction in FHB was achieved from GS 39+55 applications. Such observations are in agreement with the results obtained during this study in field trial 2 (1999/2000) where fungicides applied at GS 31+39 had no effect on FHB severity recorded at GS 85 (Table 3.6), whilst metconazole+azoxystrobin applied at GS 39+59 was the most effective treatment at reducing visual symptoms of FHB, followed by metconazole and azoxystrobin alone.

The lack of any significant difference in FHB severity at GS 85 between metconazole and azoxystrobin was probably due to the high level of natural *M. nivale* infection of wheat ears and that while metconazole reduced symptoms caused by *Fusarium* spp. this may have been masked by symptoms caused by *M. nivale*, This suggestion is supported by the quantification of *Tri*5 DNA and *M. nivale* DNA (Table 3.7) in harvested grain, which clearly indicated that metconazole was effective at reducing grain infection by trichothecene producing *Fusarium* whilst azoxystrobin reduced infection by *M. nivale*. Application of metconazole at GS 39+59 provided significant reduction of % FDK (Table 3.8) which agrees with observations recorded by Dardis and Walsh (2000). Applications of metconazole at either GS 31+39 or GS 39+59, did not provide any significant ($P>0.05$) reduction of DON in harvested grain (Table 3.8) which contrasts with the results obtained from field trial 1 (1998/1999) and with the findings of Siranidou and Buchenauer (2001) who observed that an application of metconazole reduced DON content in harvested grain by 61-69% compared to untreated control.

Such a discrepancy observed in field trial 2 might be due to an excessively high disease pressure induced by the high amount of inoculum placed into the field early in the growing season. Milus and Parsons (1994) reported no effect of a range of fungicides including tebuconazole against FHB severity and DON concentration in grain and attributed this to the high disease pressure during the two years of investigation. Another possible explanation for this discrepancy may be due to the high amount of *M. nivale* or saprophytic species like *Alternaria* spp. or *Cladosporium* spp. present on wheat plants early in the season. These could lead to elevated mycotoxin production by *Fusarium* spp. due to inter-species interactions. Liggitt *et al.* (1997) demonstrated that if species such as *Alternaria alternata* and *Cladosporium herbarum* are present on wheat ears before *Fusarium* spp., a decrease in visual symptoms of FHB occurred. It is possible, that such antagonistic interactions could lead to an increase in mycotoxin production due to induced competition. However, since Liggitt *et al.* (1997) did not record mycotoxin content in harvested grain, further work is need in this area if such a hypothesis is to be tested.

Factorial ANOVA revealed no significant effects ($P>0.05$) of the fungicides and time of their application on the incidence of *Fusarium* spp. and *M. nivale* on wheat leaves at any of the sampling stages (Appendices 8, 9 and 10). Based on these results, it could be suggested that the application of fungicides early in the growing season (GS 31-39) could have some effect on the incidence of *Fusarium* spp. on the leaves and stems of wheat plants but not on the severity of FHB, grain colonisation by *Fusarium* spp. and DON concentration in grain. However, this study also showed that in order to achieve better control of FHB, investigations on the timing of fungicides against the disease should be focused around heading (GS 59-67).

In field trial 3 (2000/2001), factorial ANOVA revealed that all fungicides provided significant control of disease severity at GS 85 when compared with untreated plots ($P<0.001$) (Table 3.9). The fungicide treatments were more effective against FHB when they were applied 2 days before or 2 days after inoculation of the wheat plots. This is in agreement with the work by Mauler-Machnik and Zahn (1994) who after applying tebuconazole either 3 days before or 3 days after the artificial inoculation of wheat ears with *Fusarium* spp. observed a 60% reduction in FHB severity when compared to untreated controls. All triazole fungicides significantly decreased DON concentration in grain when applied 5 days after inoculation but were less effective in comparison with those applied 2 days before or 2 days after inoculation. Similar effects have been observed by Homdork *et al.* (2000) who found that applications of tebuconazole against FHB caused by *F. culmorum* reduced DON in grain by 68.8% when applied 3 days before inoculation, whilst an application 5 days post-inoculation reduced DON by 53.5%. Siranidou and Buchenauer (2001) also demonstrated that tebuconazole applied 2 days before or two days after inoculation of wheat plots inoculated with *F. culmorum* resulted in a reduction of DON by 71 and 62%, respectively. This suggests that fungicide application is more effective in reducing FHB and DON when treatments are applied close to the time of fungal infection, before disease is well established.

In contrast, there have been cases where fungicides applied just before or at the time of inoculation resulted in significant increase of *Fusarium* mycotoxins in grain while providing reduction of visual symptoms. For example Gareis and Ceynova (1994) observed a 53% and 43% reduction of FHB (*F.*

culmorum) severity when a mixture of tebuconazole+triadimenol was applied either 3 hours before or 24 hours after inoculation, respectively. Although the effects of fungicide timing on disease severity were small, determination of mycotoxin contamination of harvested grain revealed that applying the fungicide mixture 3 hours before inoculation resulted in a 16-fold increase in nivalenol content whilst the application 24 hours post-inoculation increased nivalenol content 6-fold compared to untreated controls.

In this study, azoxystrobin was observed to cause an increase of *Tri5* DNA in grain when it was applied 2 days after inoculation, and a slight increase in DON concentration. However, such increases were statistically insignificant. Simpson *et al.* (2001) found that an application of azoxystrobin three days post-inoculation resulted in a significant increase in the production of DON when wheat plots were inoculated with a mixture of *Fusarium* spp. and *M. nivale.* These workers suggested that such an increase in DON was due to a reduction in competition between *Fusarium spp.* and *M. nivale*, since azoxystrobin effectively controlled grain colonisation by *M. nivale.* The regression analysis from field trial 3 (2000/01) revealed a strong relationship (r^2=68; Figure 3.2b) between *Tri5* expressing pathogens and DON concentration in grain and confirmed the previous findings from field trial 1 (1998/99), showing that an increase of trichothecene-producing *Fusarium* in grain leads to an increase in DON production.

Both Suty *et al.* (1996) and Homdork *et al.* (2000) have demonstrated that successful control of FHB can also be achieved when fungicides are applied twice, both before and after inoculation. However, due to practical and financial implications, the use of a two-spray strategy in commercial cereal production is unlikely.

The severity of sooty moulds on wheat ears shows the importance of these saprophytes as secondary colonisers after the spikelets have been bleached by the infection by head blight pathogens. With the exception of field trial 3 (2000/01), data from this study shows that the severity of sooty moulds could be directly affected by application of azoxystrobin.This is in agreement with the findings of Bertelsen *et al.* (2001) who reported that azoxystrobin inhibited spore germination and mycelial

growth of *Alternaria alternata* and *Cladosporium macrocarpum* when wheat leaves were inoculated with these saprophytic species. On the other hand, it is also possible that fungicides also reduce the incidence of ear infection by these secondary colonisers indirectly through reduction of FHB severity. It could be suggested that as FHB severity increases, there is a concomittant increase in sooty moulds incidence. However further study is necessary to demostrate this hypothesis.

During all three field trials, fungicides and their time of application had significant effect on the yield of wheat. For example in field trial 1 (1998/1999), an application of metconazole-azoxystrobin mixture increased yield by 27% (Appendix 11). All the other treatments also increased yield significantly ($P<0.001$) as well, but these were not significant. In the second field trial (1999/2000) all fungicides had a significant ($P<0.001$) effect on yield (Appendix 12). The interaction between fungicides and time of application was significant ($P=0.001$) and again showed that the metconazole-azoxystrobin mixture increased yield by 32% when applied at GS 39+59. Again there was no difference between azoxystrobin and metconazole applied alone on the yield of wheat. In agreement with our results, Siranidou and Buchenauer (2001) showed that applications of a range of fungicides including metconazole, azoxystrobin and metconazole-azoxystrobin mixture, provided an increase in yield of wheat. However, there was no significant difference between application of metconazole or azoxystrobin on yield. In field trial 2000/2001 the time of fungicide application on yield of wheat was not significant ($P>0.05$) neither was there any significant interaction between fungicides and their time of application ($P>0.05$) (Appendix 13). One possible reason for such limited effect of fungicides on yield may be due to the use of the harvesting machinery employed, which may have allowed small and shrivelled grain to be blown out of the back of the harvester thus resulting in small differences on yield between fungicide treatments.

Chapter 4

Effect of four rates of metconazole and azoxystrobin on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain

4.1 Introduction

Results from *in vitro* studies have indicated that the presence of certain fungicides can result in elevated concentrations of *Fusarium* toxins. For example, studies on the effect of the fungicide tridemorph on T-2 toxin production by *Fusarium sporotrichioides*, showed that at low concentrations (6-8 $\mu g\ g^{-1}$), the chemical produced a slight increase in the growth of the fungus and inhibited T-2 toxin and DAS (diacetoxyscripenol) production (Moss and Frank, 1985). However, at higher concentrations (30–50 $\mu g\ g^{-1}$), although the fungicide inhibited fungal growth by ca. 50%, T-2 toxin production was increased five-fold.

Under field conditions, the effect of fungicides on mycotoxin concentration is unclear. For example, during a field trial inoculated with *Fusarium culmorum*, a 16-fold increase in nivalenol concentration in wheat grains was observed after an application of the fungicide product "Matador" (tebuconazole 250 g a.i. l^{-1} + triadimenol, 125 g a.i. l^{-1}) to wheat ears, despite a reduction in disease severity (Gareis and Ceynova, 1994). In contrast, other field studies have shown that applications of propicanozole and triadimefon (Boyacioglu *et al.*, 1992), thiophanate-methyl (Ueda and Yoshizawa, 1998) and tebuconazole (Suty *et al.*, 1996) all reduced the severity of FHB and DON concentration. Applications of propiconazole were shown to significantly reduce FHB in wheat but had no effect on DON concentration (Matrin and Johnston, 1982). In recent years the application of the strobilurin fungicide azoxystrobin for FHB control has been associated with an increase in DON (Hart and Ward, 1997; Ellner and Schroer, 2000).

The aims of this study were to evaluate the effects of a range of dose rates of metconazole and azoxystrobin against (i) the development of FHB (ii) the extent by which grain is colonised by mycotoxin-producing *Fusarium* species and (iii) the concentration of DON in harvested grain, in wheat ears artificially inoculated with *F. culmorum* or *F. graminearum*

Null hypothesis tested: Azoxystrobin and metconazole do not have an effect on FHB development and deoxynivalenol concentration in grain

4.2 Materials and Methods

Plants of winter wheat (cultivar Cadenza) were grown in 15 cm plastic pots containing John Innes No.2 compost under glasshouse conditions as described in Chapter 2. When at GS 59, ears were sprayed either with metconazole or azoxystrobin at concentrations ranging from one quarter to twice the manufacturer's recommended maximum dose rate (Table 4.1). Metconazole was included in the study since it is known to be effective against *Fusarium* spp. whilst azoxystrobin has been shown to be ineffective against *Fusarium* spp. and has been observed to increase the concentration of DON in harvested grain (Hart and Ward, 1997; Ellner and Schroer, 2000). For each fungicide and dose rate, 16 replicate pots were treated. The application of sterile distilled water provided untreated controls. All fungicides were applied using a precision pot sprayer carrying Lurmark 110° flat fan nozzles (03-F110, Longstanton, Cambridge, UK) at a rate of 200 l^{-1} per ha. Inoculation of wheat plants with a conidial suspension of DON-producing isolates of either *F. culmorum* or F. *graminearum* (Table 2.1) was undertaken at mid anthesis (GS 65). Spore concentrations of 100 000 spores per ml^{-1} of water were used and ears were sprayed until run-off using a hand-held atomiser (approximately 2.5 ml per ear). Following inoculation, the ears were covered with clear polythene bags for 48 hours to provide conditions conducive to FHB development. The experiment was repeated twice and in the two experiments eight replicate pots

Table 4.1 Fungicides and their rate of use in the two glasshouse experiments

Fungicide*	Dose**	Rate***
Metconazole	2 n	180
	1 n	90
	½ n	45
	¼ n	22.5
Azoxystrobin	2 n	500
	1 n	250
	½ n	125
	¼ n	62.5

* metconazole (Caramba®) manufactured by BASF plc
azoxystrobin (Amistar®) manufactured Syngenta Crop Protection
** grams of active ingredient per hectare
*** double, full, half and quarter of the manufacturer's recommended dose rate (n)

were set-up for each treatment and arranged according to randomised block design. The assessment for the severity of FHB symptoms at GS 85, the quantification of *Tri5* DNA and DON concentration in grain were carried according to the procedures outlined in Chapter 2.

4.3 Results

Similar observations were recorded for each fungicide treatment from the two experiments although a F_{max} test revealed significant differences between the two sets of data ($P<0.001$). Data from the two experiments are, therefore, presented separately. Across all treatments, results obtained from experiment 1 revealed lower concentrations of *Tri5* DNA and DON in harvested grain when compared to results obtained from experiment 2 (see Tables 4.3 and 4.4). This may be explained by the time of year when the two experiments were performed. Experiment 1 was undertaken during September and October of 1999, whilst the repeat experiment 2 was undertaken during March and April of 2000. Although, environmental conditions were programmed to be the same for both experiments (22 ±3°C), due to the higher ambient temperatures experienced during experiment 2, temperatures in the glasshouse were observed to be as high as 30°C on some days.

The effect of fungicide treatment on the severity of FHB caused by *F. culmorum* and *F. graminearum* can be seen in Table 4.2. In both experiments, applications of either metconazole or azoxystrobin reduced significantly ($P<0.001$), the severity of disease caused by either of the two fungal pathogens when compared to the untreated controls. Of the two fungicides tested, metconazole proved to be the most effective, providing 98% (experiment 1) and 77% (experiment 2) control for plants inoculated with *F. culmorum*, and 77% (experiment 1) and 17% (experiment 2) control for plant inoculated with *F. graminearum*, when applied at the lowest dose rate. Increasing the dose rate of metconazole applied, resulted in an increase in the control of FHB achieved up to the highest dose rate of 180 g a.i.ha^{-1}, where greater than 97% control of disease was recorded whether plants were inoculated with *F. graminearum* or *F. culmorum*. Although applications of azoxystrobin reduced, significantly, the severity of FHB when compared to unsprayed controls, the percentage control achieved by this fungicide was only between 30 and 55%, even when applied at 500 g a.i.ha^{-1} (double rate). Statistical analysis revealed no evidence of a significant difference between the control of FHB achieved by each of the four dose rates of azoxystrobin used.

Table 4.2. Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on the severity of Fusarium head blight assessed 28 days after the artificial inoculation of wheat ears (cv Cadenza) at GS65 with a conidial suspension (10^5 spores per ml^{-1}) of either *F. culmorum* or *F. graminearum*. Numbers in parentheses are back-transformed means.

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Disease severity (arcsine % spikelets infected)			
			Experiment 1		Experiment 2	
F. culmorum	Control		61.01	(76.50)	45.99	(51.72)
	Metconazole	180	00.00	(00.00)	2.160	(0.140)
		90	00.00	(00.00)	1.610	(0.070)
		45	13.44	(5.400)	8.900	(2.390)
		22.5	17.13	(8.670)	20.26	(11.99)
	Azoxystrobin	500	37.98	(37.87)	29.08	(23.62)
		250	31.85	(27.84)	30.96	(26.46)
		125	32.09	(28.22)	29.24	(23.86)
		62.5	29.99	(24.98)	29.06	(23.59)
F. graminearum	Control		83.15	(98.57)	68.22	(86.23)
	Metconazole	180	6.210	(1.170)	8.520	(2.190)
		90	12.53	(4.700)	16.11	(7.690)
		45	24.77	(17.55)	16.00	(7.590)
		22.5	28.47	(22.72)	57.84	(71.66)
	Azoxystrobin	500	56.42	(69.40)	47.81	(54.89)
		250	60.25	(75.37)	64.16	(81.00)
		125	61.21	(76.80)	59.91	(74.86)
		62.5	61.79	(77.65)	57.76	(71.54)
LSD (5%) fungicide			4.36 (P<0.001)		5.64	(P<0.001)
LSD (5%) dose rate			4.78 (P<0.001)		6.18	(P<0.001)
LSD (5%) pathogen			5.85 (P<0.001)		7.57	(P<0.001)
LSD (5%) fungicide*dose rate			5.52 (P<0.001)		7.14	(P<0.001)
LSD (5%) fungicide*pathogen			6.17 (P<0.001)		7.98	(P<0.050)
LSD (5%) dose rate*pathogen			6.76 (P>0.050)		8.74	(P<0.001)
LSD (5%) fungicide*dose rate*pathogen			7.80 (P>0.050)		10.1	(P<0.050)

The competitive PCR assay employed to quantify the trichothecene-synthase gene *Tri*5, revealed that concentrations of *Tri*5 in harvested grain was significantly lower when plants were treated with either metconazole or azoxystrobin compared to the untreated controls (Table 4.3). Metconazole reduced *Tri*5 DNA concentration by 68-70% in plant inoculated with *F. culmorum* and by 70-79% in plants inoculated with *F. graminearum* over control plants, even when applied at the lowest dose rate of 22.5 g a.i.ha^{-1} (quarter rate). Increasing the dose rate of metconazole resulted in a concomitant decrease in *Tri*5 concentration until at 180 g a.i.ha^{-1}; a reduction of between 97 and 100% was achieved for experiments 1 and 2, respectively. Azoxystrobin proved less effective at reducing *Tri*5 concentration than metconazole, with reductions of between 20 and 40% for plants inoculated with *F. culmorum*, and between 20 and 52 % for plants inoculated with *F. graminearum* being observed in the respective experiments, where the highest dose rate of 500 g a.i.ha^{-1} was applied.

The results for the concentration of DON recorded in harvested grain are presented in Table 4.4. Results recorded for each treatment were similar to those seen with the concentration of *Tri*5 in harvested grain. Although both fungicides reduced, significantly, the concentration of DON compared to control plants, metconazole proved the most effective, reducing DON concentration by greater than 95% when applied at dose rates above 45 g a.i.ha^{-1} (half rate), irrespective of the pathogen used. Dose rates of half rate or greater of azoxystrobin were only able to reduce DON concentrations by 20-75% depending on the pathogen used.

Regression analysis revealed a significant and strong relationship between the concentration of the *Tri*5 gene in harvested grain and DON concentration (Figure 4.1). In both experiments, and for each fungicide treatment, it was seen that an increase in *Tri*5 concentration resulted in a concomitant increase in DON concentration. Comparison of linear regression models showed that in both experiments, the relationship between *Tri*5 and DON concentration in grain harvested from ears treated with azoxystrobin was not significantly different to that obtained for untreated controls. However, for plants treated with metconazole, the relationship between *Tri*5 and DON was significantly different to that obtained for untreated and azoxystrobin treated plants with a lower concentration of DON per copy of *Tri*5 DNA.

Table 4.3 Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on the quantity of *Tri*5 DNA in harvested grain after artificial inoculation of wheat ears (cv Cadenza) at GS65 with a conidial suspension (10^5 spores per ml^{-1}) of either *F. culmorum* or *F. graminearum*. Number in parentheses are back-transformed means

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Log_{10} (*Tri*5 DNA+1) (pg ng^{-1} total DNA)	
			Experiment 1	Experiment 2
F. culmorum	Control		0.552 (3.566)	1.532 (34.04)
	Metconazole	180	0.000 (1.000)	0.065 (1.161)
		90	0.000 (1.000)	0.102 (1.264)
		45	0.029 (1.069)	0.393 (2.471)
		22.5	0.056 (1.137)	0.999 (9.977)
	Azoxystrobin	500	0.330 (2.142)	1.222 (16.67)
		250	0.213 (1.635)	1.346 (22.18)
		125	0.259 (1.816)	1.417 (26.12)
		62.5	0.204 (1.602)	1.263 (18.32)
F. graminearum	Control		0.982 (9.598)	2.227 (168.6)
	Metconazole	180	0.062 (1.155)	0.457 (2.864)
		90	0.066 (1.164)	0.647 (4.436)
		45	0.150 (1.412)	0.565 (3.672)
		22.5	0.299 (1.993)	1.709 (51.16)
	Azoxystrobin	500	0.470 (2.957)	1.739 (62.08)
		250	0.541 (3.477)	2.039 (109.3)
		125	0.505 (3.201)	1.942 (87.49)
		62.5	0.598 (3.968)	1.976 (94.62)
LSD (5%) fungicide			0.065 (P<0.001)	0.194 (P<0.001)
LSD (5%) dose rate			0.071 (P<0.050)	0.211 (P<0.001)
LSD (5%) pathogen			0.087 (P<0.001)	0.259 (P<0.001)
LSD (5%) fungicide*dose rate			0.082 (P>0.050)	0.244 (P<0.001)
LSD (5%) fungicide*pathogen			0.092 (P<0.001)	0.273 (P>0.050)
LSD (5%) dose rate*pathogen			0.101 (P<0.050)	0.299 (P<0.050)
LSD (5%) fungicide*dose rate*pathogen			0.116 (P>0.050)	0.346 (P>0.050)

Table 4.4 Effect of metconazole and azoxystrobin applied at four dose rates at GS59 on deoxynivalenol (DON) concentration in harvested grain after artificial inoculation of wheat ears (cv Cadenza) at GS65 with a conidial suspension (10^5 spores per ml^{-1}) of either *F. culmorum* or *F. graminearum*

Pathogen	Fungicide	Dose rate (g a.i. ha^{-1})	Deoxynivalenol concentration (mg kg^{-1})	
			Experiment 1	Experiment 2
F. culmorum	Control		25.96	33.28
	Metconazole	180	00.00	00.00
		90	00.00	00.10
		45	0.310	1.340
		22.5	1.410	11.87
	Azoxystrobin	500	10.46	19.61
		250	6.860	25.46
		125	6.610	21.03
		62.5	5.710	18.12
F. graminearum	Control		55.15	63.56
	Metconazole	180	0.350	0.450
		90	1.170	1.950
		45	8.810	2.420
		22.5	8.330	37.70
	Azoxystrobin	500	25.91	36.78
		250	28.77	47.32
		125	31.75	51.03
		62.5	34.62	48.26
LSD (5%) fungicide			3.53 (P<0.001)	5.74 (P<0.001)
LSD (5%) dose rate			3.87 (P>0.050)	6.29 (P<0.001)
LSD (5%) pathogen			4.74 (P<0.001)	7.70 (P<0.001)
LSD (5%) fungicide*dose rate			4.74 (P>0.050)	7.26 (P<0.001)
LSD (5%) fungicide*pathogen			5.00 (P<0.001)	8.12 (P<0.001)
LSD (5%) dose rate*pathogen			5.47 (P<0.050)	8.89 (P<0.050)
LSD (5%) fungicide*dose rate*pathogen			6.32 (P>0.050)	10.2 (P>0.050)

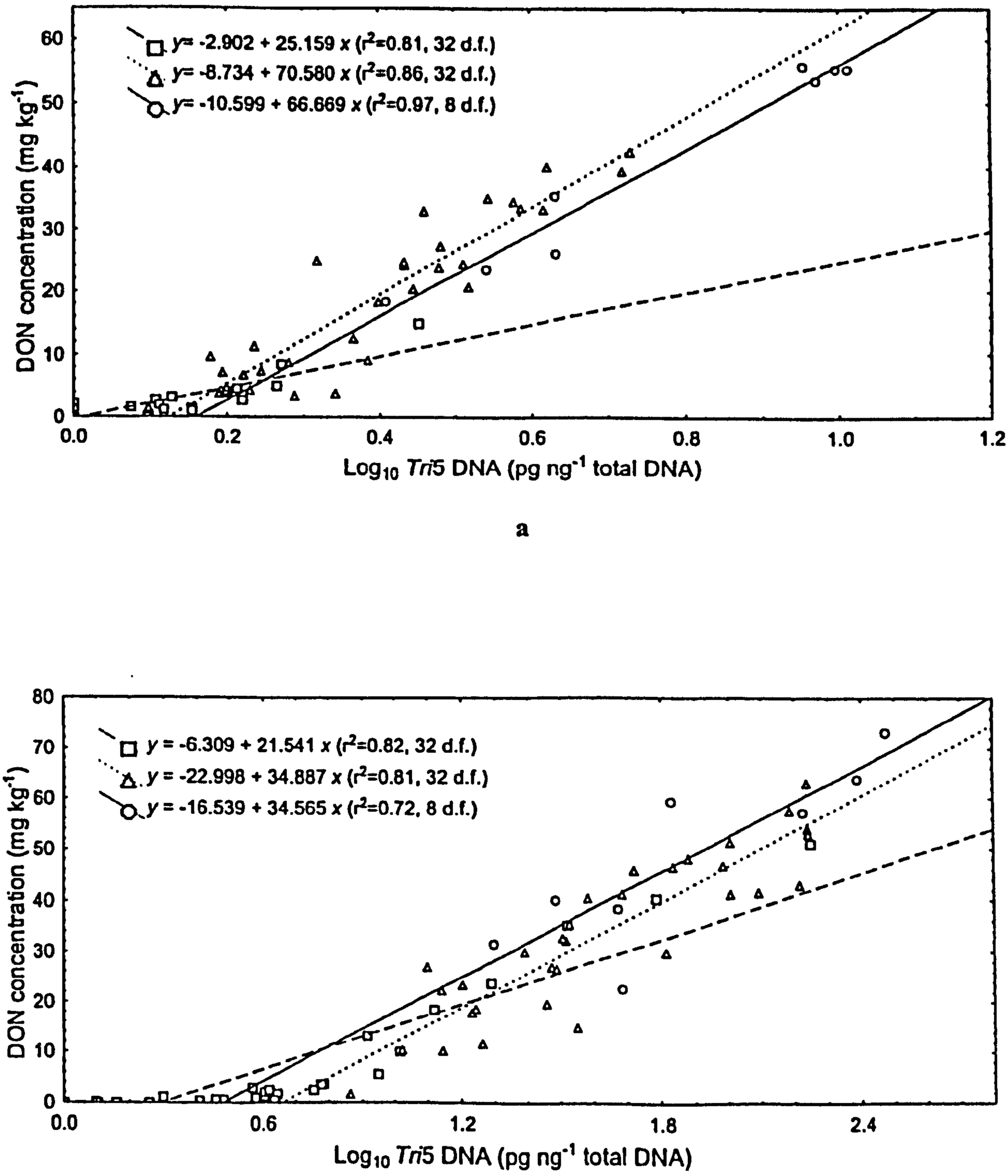


Figure 4.1. Relationship between quantity of *Tri5* DNA and DON concentration in grain of winter wheat (cv. Cadenza) harvested from ears treated with a range of dose rates of metconazole (○), azoxystrobin (□) or untreated (Δ) in experiment 1 (a) and experiment 2 (b).

4.4 Discussion

Results from both experiments indicate that DON concentration present in harvested grain was strongly influenced by the concentration of the *Tri*5 gene in grain as determined by the competitive PCR assay. For plants sprayed with either azoxystrobin or metconazole, fitting a simple linear model to *Tri*5 and DON data accounted for between 81% and 86% of variance, depending on the fungicide and experiment (Figure 4.1). Such strong relationships between *Tri*5 and DON concentration suggest that in this study, the fungicide treatments tested did not elevate DON concentration over and above that which was determined by the quantity of trichothecene-producing *Fusarium* present in grain. Had an individual treatment resulted in a cluster of points above the regression line, this would have suggested that such a treatment increased the concentration of DON produced per *Tri*5 copy. The lack of any obvious cluster indicates that neither azoxystrobin nor metconazole influenced DON concentration within grain other than by altering the amount of trichothecene-producing *Fusarium* present. These findings support those recorded under field conditions in Chapter 3 where grain samples taken from the field trials revealed that DON concentration was strongly correlated to the concentration of *Tri*5 DNA.

Applications of azoxystrobin applied two days after the artificial inoculation of wheat ears with a mixture of head blight causing pathogens, including *F. culmorum* and *F. avenaceum*, have been observed to increased DON concentration per unit of pathogen DNA present in wheat grain (Simpson *et al.*, 2001). Other field studies have also revealed an increase on DON concentration following an application of azoxystrobin when compared to unsprayed controls. Results from this study are contrary to these findings. By using a regression analysis approach, it can be seen in this study, that applications of azoxystrobin even at dose rates double that of the manufacturer's recommendations, failed to indicate an elevation of DON concentration over and above that which is determined by the quantity of trichothecene-producing pathogens colonising the grain.

The higher concentration of *Tri5* DNA and DON found in grain across all treatments in experiment 2 may be explained by the fact that the warmer environmental conditions were experienced during this experiment and these may have been more favourable for infection and colonisation of grain by *F.*

culmorum and *F. graminearum*. Ruy and Bullerman (1999), observed that *F. graminearum* produced greater quantities of DON and zearalenone when incubated under temperatures cycling between 15 and 30° C than under temperature cycling between 5 and 30°C and 10 and 25°C. It is possible, therefore, that the higher temperatures experienced during experiment 2, may have played some role in the slight elevation in DON concentration recorded when azoxystrobin was applied at the highest dose rate. Further studies on the effect of temperature and its interaction with fungicides on DON accumulation are, therefore, recommended.

The greater reduction in FHB, *Tri*5 DNA and DON in grain achieved by metconazole indicated that this fungicide was very effective against both *F. culmorum* and *F. graminearum*. Such observations are in agreement with those of other studies (Jennings *et al.*, 2001; Mathies and Buchenauer, 2000) where the triazole fungicides metconazole and tebuconazole were shown to be effective at reducing both FHB and DON. Dardis and Walsh (2000) also showed that metconazole was the most effective of a range of fungicides tested against FHB caused by *F. culmorum*.

Although azoxystrobin reduced significantly, FHB, *Tri*5 and DON when compared to unsprayed controls, it was far less effective than metconazole, and unlike metconazole, no significant difference in the level of control was observed between the dose rates used. Such observations are consistent with those of other field studies where applications of azoxystrobin were shown to reduce both FHB and DON concentration in wheat when compared to unsprayed controls (Milus and Weight, 1998; Jones, 2000). The relatively poor efficacy of azoxystrobin against FHB cause by *F. culmorum* and *F. graminearum* in this study supports previous observations that although the fungicide is effective against FHB caused by *Microdochium nivale*, it has very limited activity against *Fusarium* spp. (Simpson *et al.*, 2001). Jones *et al.* (2001) compared the effect of azoxystrobin applied to winter wheat plots at 62.5, 125, 187.5 and 250 g a.i. ha^{-1} against the severity of a range of diseases. Plotting disease severity against fungicide dose rate allowed dose rate response curves to be drawn for each of the diseases assessed. Dose response curves clearly highlighted a rate response for the majority of diseases, in the case of *Septoria tritici*, the rate response curve had levelled out and was almost horizontal between the four dose rates of azoxystrobin tested. This would suggest that lower dose

rates of azoxystrobin would need to be tested in order to determine a dose rate response for *S. tritici*. Since FHB, *Tri5* and DON did not respond to dose rate of azoxystrobin applied in these studies, it would appear that the four dose rates used here were above those, which could allow a rate response to be determined.

Given the food safety issues associated with wheat grain contaminated with trichothecene mycotoxins, it is proposed that fungicides provide a valuable tool for reducing DON concentration in wheat by effectively reducing the colonisation of grain by trichothecene-producing *Fusarium* species and that they pose no significant threat towards the elevation of DON accumulation via possible fungicidal stress influences.

Chapter 5

Studies on the interaction between fungicides, saprophytic microflora and *M. nivale* on Fusarium head blight development and deoxynivalenol concentration in grain caused by *F. culmorum*

Introduction

The discrepancy between the fungicide performance against FHB development and mycotoxin accumulation in grain may be explained by several factors including formulation of the products, time of the fungicide application, and differences in varietal resistance. In addition, the presence of saprophytic fungi such as *Alternaria* spp., *Botrytis* spp. and *Cladosporium* spp. which also colonise cereal ears may also influence FHB development and mycotoxin accumulation in grain and field performance of fungicides. For example, Bateman (1979) studied the relationship between saprophytic species and *M. nivale* on wheat ears and wheat seed. Grain collected from wheat ears, inoculated with *Alternaria alternata*, *Cladosporium* spp. and S*porobolobomyces* spp. at anthesis followed by inoculation with *M. nivale*, yielded significantly less *M. nivale* in comparison with saprophyte free ears. Similar results have been shown by Liggitt *et al.* (1997), during glasshouse studies where wheat plants were inoculated with either, *A. alternata*, *Botrytis cinerea* or *Cladosporium herbarum* at GS 59 followed by inoculation with *F. culmorum* at GS 65. All these saprophytes reduced FHB severity between 46% and 78% compared to plants inoculated only with *F. culmorum* at GS 65 (control). When these species were introduced to wheat ears after inoculation with *F. culmorum, B. cinerea* and *C. herbarum* had no effect on FHB severity, whilst *A. alternata* significantly increased disease symptoms by 49% over the control treatment. Liggitt *et al.* (1997) also demonstrated during *in vitro* work, that fungicides had differential effect on the species used in their study. For example pyrimethanil, reduced mycelial growth of *A. alternata* by up to 92%, but failed to reduce growth of *F. culmorum*, *B. cinerea* or *C. herbarum* by more than 27%. Conversely, flusilazole provided up to 90% reduction of *F. culmorum* but failed to reduce mycelial growth of *B. cinerea* and *C. herbarum* by more than 59%. Bertelsen *et al.* (2001) reported that azoxystrobin inhibited spore germination and mycelial growth of *A. alternata* and *C. macrocarpum* when wheat leaves were inoculated with these saprophytic species.

Such findings may suggest that removal of the non-toxin producing fungi from the ear by specific fungicides may have the overall effect of diminishing the inter-specific competition and result in an

increased FHB development and mycotoxin production due to the growth of toxigenic *Fusarium* spp. that are less sensitive to those fungicides.

A study by Reinecke *et al.* (1979) on the effect of MBC fungicides on the antagonism between fungi involved in the foot rot complex of cereals, indicated that in plots treated with a range of MBC (mainly benodanil) fungicides, reduced the incidence of eyespot (*Pseudocercosporella herpotrichoides)* symptoms by ca. 50% but increased the incidence of fusarium foot rot (*Fusarium* spp.) and sharp eyespot (*Rhizoctonia cerealis).* Jennings *et al.* (2000) suggested that applications of azoxystrobin might impose a significant effect on the interaction between *F. culmorum* and *M. nivale* and on the consequent mycotoxin production. In two years of field studies, applications of either tebuconazole, metconazole or carbendazim two days after wheat plots were inoculated at GS 65 with conidial suspension of *F. avenaceum*, *F. culmorum* and *M. nivale*, significantly reduced the amount of *Fusarium* spp. and DON quantified in harvested wheat grain. Applications of these fungicides also resulted in an increase in *M. nivale* colonising grain. In the first year of their studies, azoxystrobin effectively controlled *M. nivale* which in turn reduced the competition with *Fusarium* species and resulted in increased DON in grain by ca. 67%. In the second year, *M. nivale* was not present on wheat ears and no increase of DON in grain after treatment with azoxystrobin was detected. More recently, Simpson *et al.* (2001) associated applications of azoxystrobin with an increase in grain DON content. In a field trial artificially inoculated with a mixture of *F. avenaceum*, *F. culmorum* and *M. nivale* at GS 65, an application of azoxystrobin 3 days post-inoculation resulted in an increase in DON concentration of harvested grain by ca. 56% when compared to control plots. The quantification of *F. culmorum* DNA did not indicate an increase of this species in grain, although *M. nivale* DNA was significantly reduced when azoxystrobin was applied.

In an investigation on the interaction between *Bipolaris sorokiniana* and *F. graminearum* on the ears of barley, Tekauz and McCallum (2000a) found a 91% reduction in FHB symptoms when *B. sorokiniana* was present on barley ears before the introduction of *F. graminearum.* The same authors (Tekauz and McCallum, 2000b) explained the higher resistance of two-row barley cultivars

to FHB compared to six-row cultivars as partly due to the high incidence of *Cochliobolus sativus* colonising the ears of two-row varieties. These workers found that most two-row barley cultivars usually had higher levels of seed-borne *C. sativus* and suggested that this species may have reduced FHB levels and suppressed the isolation of *Fusarium* from infected seed. However, there were no reports on the effect of the presence of *C. sativus* on mycotoxin concentration.

Although saprophytic fungi, *M. nivale* or other species interact with toxin-producing head blight pathogens, there is a little experimental evidence to demonstrate that these interactions could explain the poor field fungicide performance or the unexpected increase of *Fusarium* toxins in harvested grain.

The aim of this study was to (i) evaluate the role of *A. tenuissima*, *C. herbarum* and *M. nivale* in FHB development, DON production in wheat and (ii) to evaluate the performance of azoxystrobin and metconazole against FHB and DON production caused by *F. culmorum* in the presence of *A. tenuissima*, *C. herbarum* and *M. nivale*.

Null hypothesis tested:

1. Saprophytic species and *M. nivale* have no effect on FHB development and mycotoxin accumulation in grain of wheat.
2. Fungicides do not affect interaction between *Fusarium* spp. and *M. nivale* or saprophytes.

5.2 Materials and methods

Three glasshouse experiments were undertaken which involved the production of potted winter wheat plants (cultivar Cadenza) produced according to the procedures described in Chapter 2.

5.2.1 Experiment 1. Effect of *Alternaria tenuissima* on the severity of FHB and DON accumulation caused by *F. culmorum* in wheat

The experimental approach employed involved the artificial inoculation of all wheat ears with a conidial suspension of *F. culmorum* at GS 65. Conidial suspensions of all other species involved in

these studies were as described for *Fusarium* spp. in Chapter 2. Twenty one pots were artificially inoculated with a conidial suspension of *A. tenuissima* (150,000 spores per ml of water) at GS 57 (2.5 ml spore suspension per ear). Of these, seven pots received an application of metconazole at GS59 whilst another seven pots received an application of azoxystrobin at GS59. The remaining seven pots received no fungicide treatment. A further 21 pots were also artificially inoculated with *A. tenuissima* 24 hours after inoculation with *F. culmorum*. Of these, seven were sprayed with metconazole and seven were sprayed with azoxystrobin at GS59 (Table 5.1) whilst the remaining seven pots were left unsprayed. In all cases, ears were covered with clear polythene bags for 24 hours following inoculation to provide conditions conducive to FHB development. Pots were arranged on a bench according to a randomised block design and the glasshouse maintained at 22 ± 3^{O}C and a 16 hour photoperiod.

5.2.2 Experiment 2 Effect of *Cladosporium herbarum* on the severity of FHB and DON accumulation caused by *F. culmorum* in wheat

The experimental procedure was identical to that described above for *A. tenuissima* (see Table 5.2).

5.2.3 Experiment 3. Effect of *Microdochium nivale* on the severity of FHB and DON accumulation caused by *F. culmorum* in wheat

The experimental procedure was identical to that described above for *A. tenuissima and C. herbarum* with the exception that only six replicate pots were used per treatment (see Table 5.3)

In each experiment, all ears were assessed for the severity of FHB symptoms at GS 85. The total number of spikelets and the number showing necrosis or bleaching were recorded to give the percentage of infected spikelets. The quantification of *Tri5* DNA, *M. nivale* DNA and deoxynivalenol concentration in grain were as described in Chapter 2.

Table 5.1 Treatment structure for glasshouse experiment 1.

Treatment number	Growth stage of wheat plants when inoculation with the different species or fungicide application was carried out			
	57	59	65	65+
1 Control			*F. culmorum*	
2		metconazole	*F. culmorum*	
3		azoxystrobin	*F. culmorum*	
4	*A .tenuissima*		*F .culmorum*	
5			*F. culmorum*	*A. tenuissima*
6	*A .tenuissima*	metconazole	*F. culmorum*	
7	*A. tenuissima*	azoxystrobin	*F. culmorum*	
8		metconazole	*F. culmorum*	*A. tenuissima*
9		azoxystrobin	*F. culmorum*	*A. tenuissima*

Table 5.2 Treatment structure for glasshouse experiment 2.

Treatment number	Growth stage of wheat plants when inoculation with the different species or fungicide application was carried out			
	57	59	65	65+
1 Control			*F. culmorum*	
2		metconazole	*F. culmorum*	
3		azoxystrobin	*F. culmorum*	
4	*C. herbarum*		*F. culmorum*	
5			*F. culmorum*	*C. herbarum*
6	*C. herbarum*	metconazole	*F. culmorum*	
7	*C. herbarum*	azoxystrobin	*F. culmorum*	
8		metconazole	*F. culmorum*	*C. herbarum*
9		azoxystrobin	*F. culmorum*	*C. herbarum*

Table 5.3 Treatment structure for glasshouse experiment 3.

Treatment number	Growth stage of wheat plants when inoculation with the different species or fungicide application was carried out			
	57	59	65	65+
1 Control			*F. culmorum*	
2		metconazole	*F. culmorum*	
3		azoxystrobin	*F. culmorum*	
4	*M. nivale*		*F. culmorum*	
5			*F. culmorum*	*M. nivale*
6	*M. nivale*	metconazole	*F. culmorum*	
7	*M. nivale*	azoxystrobin	*F. culmorum*	
8		metconazole	*F. culmorum*	*M. nivale*
9		azoxystrobin	*F. culmorum*	*M. nivale*

5.3 Results

5.3.1 Experiment 1.

Factorial ANOVA of data collected from the assessment of FHB severity at GS 85 revealed that the overall effect of azoxystrobin on FHB was not significantly different compared to the control treatment, whilst metconazole provided significant ($P>0.001$) reduction of FHB. Analysis showed that when *A. tenuissima* was introduced on wheat ears (GS 57) before the introduction of *F. culmorum* (GS 65), there was an increase of FHB severity by 51% in comparison with the control treatment. When *A. tenuissima* was introduced to wheat ears 24 hours after *F. culmorum*, 40% less FHB symptom development than the control was recorded (Table 5.4). The quantification of *Tri5* DNA in harvested grain presented in Table 5.4 showed that in the absence of fungicides, plants inoculated with *A. tenuissima* at GS 57 significantly increased the quantity of trichothecene-producing species in wheat grain ($P<0.01$) compared to the control treatment. Application of metconazole, either before or after inoculation of plants with *A. tenuissima*, reduced significantly *Tri5* DNA in grain. Applications of azoxystrobin after the inoculation of wheat plants with *A. tenuissima* resulted in an increase of *Tri5* DNA in grain, but it was not significant in comparison with untreated control.

The introduction of *A. tenuissima* before the inoculation of ears with *F. culmorum* resulted in an increase in the concentration of DON in grain by 28% when compared to the control treatment. Applying azoxystrobin after the introduction of *A. tenuissima* to wheat plants at GS 57 or 24 hours after the introduction of *F. culmorum*, did not increase DON content in grain. There was no significant interaction between the time of inoculation of wheat plants with *A. tenuissima* and the fungicides metconazole or azoxystrobin ($P>0.05$) (Table 5.4). Regression analysis revealed a strong and significant relationship between *Tri5* DNA and DON concentration in grain ($r^2=55$, $P<0.001$) (Figure 5.1).

5.3.2 Experiment 2.

Assessment of FHB severity at GS 85 indicated that inoculation of wheat ears with *C. herbarum* alone at GS 57, before the introduction of *F. culmorum*, significantly increased FHB severity by 60% when compared with the control treatment. When *C. herbarum* was present on wheat ears 24 hours after inoculation with *F. culmorum* at GS 65, FHB was reduced by 50% over the control treatment (Table 5.5). Applying azoxystrobin either before of after the introduction of *C. herbarum* to wheat ears had no significant effect on FHB development ($P>0.05$) in comparison with the control treatment. As presented in Table 5.5, *C. herbarum* did not have any effect on the *Tri5* DNA content in grain when inoculated either before or after the introduction of *F. culmorum* to wheat ears or in combinations with metconazole or azoxystrobin ($P>0.05$). ANOVA revealed that when *C. herbarum* was introduced to wheat ears at GS 57 before the introduction of *F. culmorum*, DON concentration in grain was significantly ($P<0.05$) increased by 34% in comparison with control treatment (Table 5.5). An application of metconazole either before or after the inoculation of wheat plants with *C. herbarum* provided a significant reduction of DON concentration in grain ($P<0.05$). Azoxystrobin had no significant effect on DON in grain when compared with control treatment. However, treatment with azoxystrobin significantly reduced DON in grain by 26-47% when compared to the treatment when *C. herbarum* was introduced before *F. culmorum* in the absence of fungicides. Regression analysis revealed a strong and significant relationship between *Tri5* DNA and DON concentration in grain ($r^2=78$, $P<0.001$) (Figure 5.2).

Table 5.4 Effect of artificial inoculation of ears of winter wheat (cv Cadenza) with *A. tenuissima* at GS 57 or 65+ (24 hours after inoculation with *F. culmorum*) alone or in combination with metconazole or azoxystrobin applied at GS 59 on the severity of Fusarium head blight (FHB) assessed at GS 80, *Tri*5 DNA quantity and DON concentration in grain. Numbers in parentheses are back-transformed means

Time of inoculation or fungicide application				Arcsine % spikelets infected	Log_{10} *Tri*5 DNA pg ng^{-1} of total DNA	DON mg kg^{-1}
GS 57	GS 59	GS 65	GS 65+			
Control		*F. c.***		25.22 (18.15)	1.52 (033.1)	56.6
*A. t.**		*F. c.*		31.60 (27.45)	2.09 (123.0)	72.7
		F. c.	*A. t.*	18.72 (10.30)	1.38 (023.9)	59.9
	metconazole†	*F. c*		06.68 (01.35) †	0.63 (004.2) †	06.7†
	azoxystrobin†	*F. c*		25.98 (19.18) †	1.73 (053.7) †	49.3†
A. t.	metconazole	*F. c.*		16.64 (08.20)	0.96 (009.1)	30.0
A. t.	azoxystrobin	*F. c.*		31.62 (27.48)	1.91 (081.2)	48.4
	metconazole	*F. c.*	*A. t.*	07.57 (01.73)	0.87 (007.4)	6.90
	azoxystrobin	*F. c.*	*A. t.*	20.66 (12.44)	0.74 (005.4)	35.8
LSD (5%) Fungicide				6.46 (P<0.001) CV=31.7 %	0.43 (P<0.001) CV=33.9 %	27.1 (P<0.001) CV=65.2 %
LSD (5%) Time				6.09 (P<0.001) CV=31.7 %	0.40 (P<0.001) CV=33.9 %	25.5 (P>0.05) CV=65.2 %
LSD (5%) Fungicide *time				7.45 (P>0.05) CV=31.7 %	0.49 (P<0.01) CV=33.9 %	31.3 (P>0.05) CV=65.2 %

A. tenuissima*, * F. culmorum*, † not included in the analysis

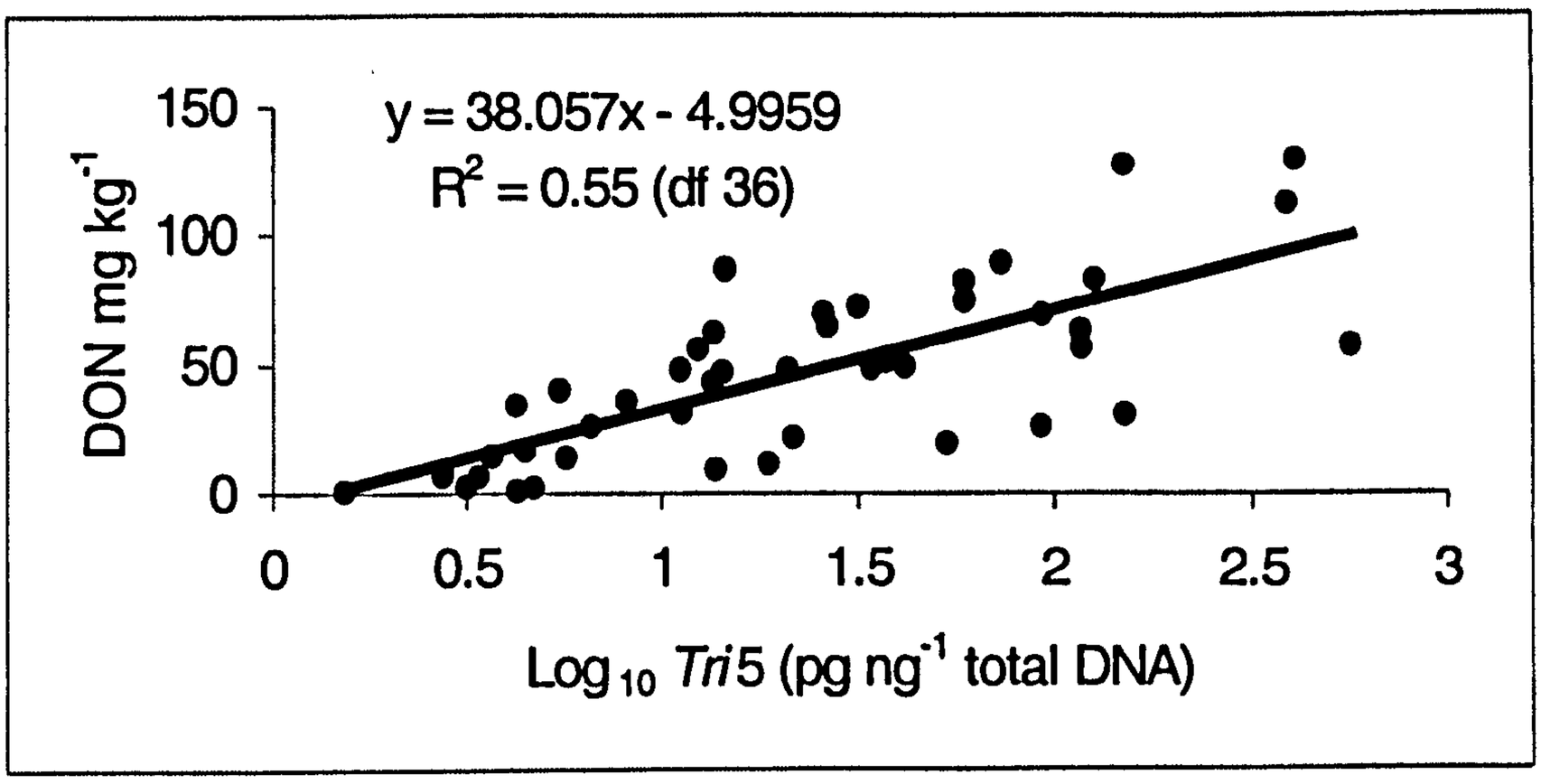


Figure 5.1 Relationship between quantity of *Tri*5 DNA and DON concentration in grain of winter wheat (cultivar Cadenza) in glasshouse experiment 1.

5.3.3 Experiment 3.

The effect of different times of *M. nivale* introduction to wheat ears and fungicides on FHB development caused by *F. culmorum* is present on Table 5.6. Inoculation of wheat ears at GS 57 with *M. nivale*, before the introduction of *F. culmorum*, significantly (P=0.001) increased the severity of FHB by 265% in comparison with untreated control, whilst introducing *M. nivale* to ears 24 hours after inoculation with *F. culmorum* at GS 65, had no significant effect on FHB development. Although an application of azoxystrobin at GS 59, after the inoculation of wheat ears with *M. nivale* at GS 57 or at 65+, resulted in an increase of FHB, although it was proved not statistically different from the control. When these treatments were compared with that where *M. nivale* was introduced at GS 57 and *F. culmorum* at GS 65 a significant reduction of FHB (73%) was observed. Analysis of *Tri*5 DNA in grain revealed that an application of metconazole, either before or after the inoculation of ears with *M. nivale*, provided significant reduction of *F. culmorum* in grain in comparison with control treatment, whilst inoculation of plants with *M. nivale* alone or in combination with azoxystrobin, had no effect on *Tri*5 DNA content in grain. Factorial ANOVA revealed no significant interaction between the time of inoculation with *M. nivale* and fungicides (Table 5.6). Quantification of *M. nivale* DNA in grain showed that the highest amount of this fungus was detected when plants were inoculated with *M. nivale* at GS 57 or in combination with metconazole. Treatments containing azoxystrobin were the most efficacious at reducing *M. nivale* in grain (Appendix 14). Factorial ANOVA revealed that when *M. nivale* was introduced to wheat ears before *F. culmorum*, DON concentration in grain increased significantly by 151% when compared with control treatment. There was no effect on DON concentration in grain when *M. nivale* was introduced 24 hours after inoculation of plants with *F. culmorum*. When azoxystrobin was applied after the introduction of *M. nivale* at GS 57, and before the introduction of this fungus on ears after GS 65+, DON content in grain increased by 56% and 30%, respectively. However, this increase was not significantly different from the control treatment. When these treatments were compared to that when *M. nivale* was introduced onto wheat ears at GS 57 before *F. culmorum*, a significant decrease in DON by 38-48% in grain was detected. When metconazole was applied before the introduction of *M. nivale* on wheat ears a significant reduction of DON by 80% was observed.

Table 5.5 Effect of artificial inoculation of ears of winter wheat (cv Cadenza) with *Cladosporium herbarum* at GS 57 or 65+ (24 hours after inoculation with *F. culmorum*) alone or in combination with metconazole or azoxystrobin applied at GS 59 on the severity of Fusarium head blight (FHB) assessed at GS 80, *Tri*5 DNA quantity and DON concentration in grain. Numbers in parentheses are back-transformed means

Time of inoculation or fungicide application				Arcsine % spikelets infected	Log_{10} *Tri*5 DNA pg ng^{-1} of total DNA	DON mg kg^{-1}
GS 57	GS 59	GS 65	GS 65+			
Control		*F. c.***		25.22 (18.15)	1.52 (33.11)	56.6
*C. h.**		*F. c.*		32.65 (29.10)	1.24 (17.37)	76.1
		F. c.	*C.h.*	17.62 (09.16)	0.77 (05.88)	38.2
	metconazole†	*F. c*		06.68 (01.35) †	0.63 (04.26) †	06.7†
	azoxystrobin†	*F. c*		25.98 (19.18) †	1.73 (53.70) †	49.3†
C. h.	metconazole	*F. c.*		10.79 (03.50)	0.50 (03.16)	15.2
C. h.	azoxystrobin	*F. c.*		26.55 (19.97)	1.03 (10.71)	56.3
	metconazole	*F. c.*	*C. h.*	07.29 (01.61)	0.51 (03.23)	9.30
	azoxystrobin	*F. c.*	*C. h.*	22.42 (14.54)	0.89 (07.76)	40.8
LSD (5%) Fungicide				5.15 (P<0.001) CV=26.9 %	0.30 (P<0.001) CV=34.5 %	15.43 (P<0.001) CV=39.3 %
LSD (5%) Time				4.85 (P<0.001) CV=26.9 %	0.28 (P<0.05) CV=34.5 %	14.55 (P>0.001) CV=39.3 %
LSD (5%) Fungicide *time				5.94 (P<0.05) CV=26.9 %	0.34 (P>0.05) CV=34.5 %	17.82 (P<0.05) CV= 39.3 %

**C. herbarum*, ***F. culmorum*, † not included in the analysis

Table 5.6 Effect of artificial inoculation of ears of winter wheat (cv Cadenza) with *Microdochium nivale* at GS 57 or 65+ (24 hours after inoculation with *F. culmorum*) alone or in combination with metconazole or azoxystrobin applied at GS 59 on the severity of Fusarium head blight (FHB) assessed at GS 80, *Tri*5 DNA quantity and DON concentration in grain. Numbers in parentheses are back-transformed means

Time of inoculation or fungicide application				Arcsine % spikelets infected	Log_{10} *Tri*5 DNA pg ng^{-1} of total DNA	DON mg kg^{-1}
GS 57	GS 59	GS 65	GS 65+			
Control		*F. c.*		18.7 (10.27)	0.53 (03.38)	16.2
M. n.		*F. c.*		37.8 (37.56)	0.95 (08.91)	40.8
		F. c.	*M. n.*	14.6 (06.35)	0.45 (02.81)	18.1
	metconazole†	*F. c*		12.2 (04.53)†	0.26 (01.81)†	07.9†
	azoxystrobin†	*F. c*		18.7 (10.33)†	0.49 (03.09)†	15.4†
M. n.	metconazole	*F. c.*		13.4 (05.37)	0.42 (02.63)	12.3
M. n.	azoxystrobin	*F. c.*		22.9 (10.51)	0.72 (05.24)	25.4
	metconazole	*F. c.*	*M. n.*	8.90 (02.39)	0.14 (01.38)	3.20
	azoxystrobin	*F. c.*	*M. n.*	18.9 (10.49)	0.48 (03.01)	21.3
LSD (5%) Fungicide				6.65 (P<0.001) CV=33.6 %	0.20 P<0.001) CV=38.1 %	8.0 (P<0.001) CV=40.2 %
LSD (5%) Time				6.27 (P<0.001) CV=33.6 %	0.19 (P<0.001) CV=38.1 %	7.5 (P>0.001) CV=40.2 %
LSD (5%) Fungicide *time				7.68 (P=0.001) CV=33.6 %	0.23 (P>0.05) CV=38.1 %	9.2 (P<0.05) CV= 40.2 %

**M. nivale*, ** *F. culmorum*, † not included in the analysis

Regression analysis of data obtained for FHB severity, *Tri5* DNA and DON revealed strong and significant ($P<0.001$) relationships between FHB severity and DON content ($r^2= 0.72$) FHB severity and *Tri5* DNA ($r^2= 0.75$) and *Tri5* DNA and DON content ($r^2= 0.81$)(Figure 5.3; 5.4; 5.5). No significant relationship between *M. nivale* DNA and DON or *M. nivale* DNA and FHB severity was observed suggesting that disease symptoms of FHB in this trial were caused only by *F. culmorum*.

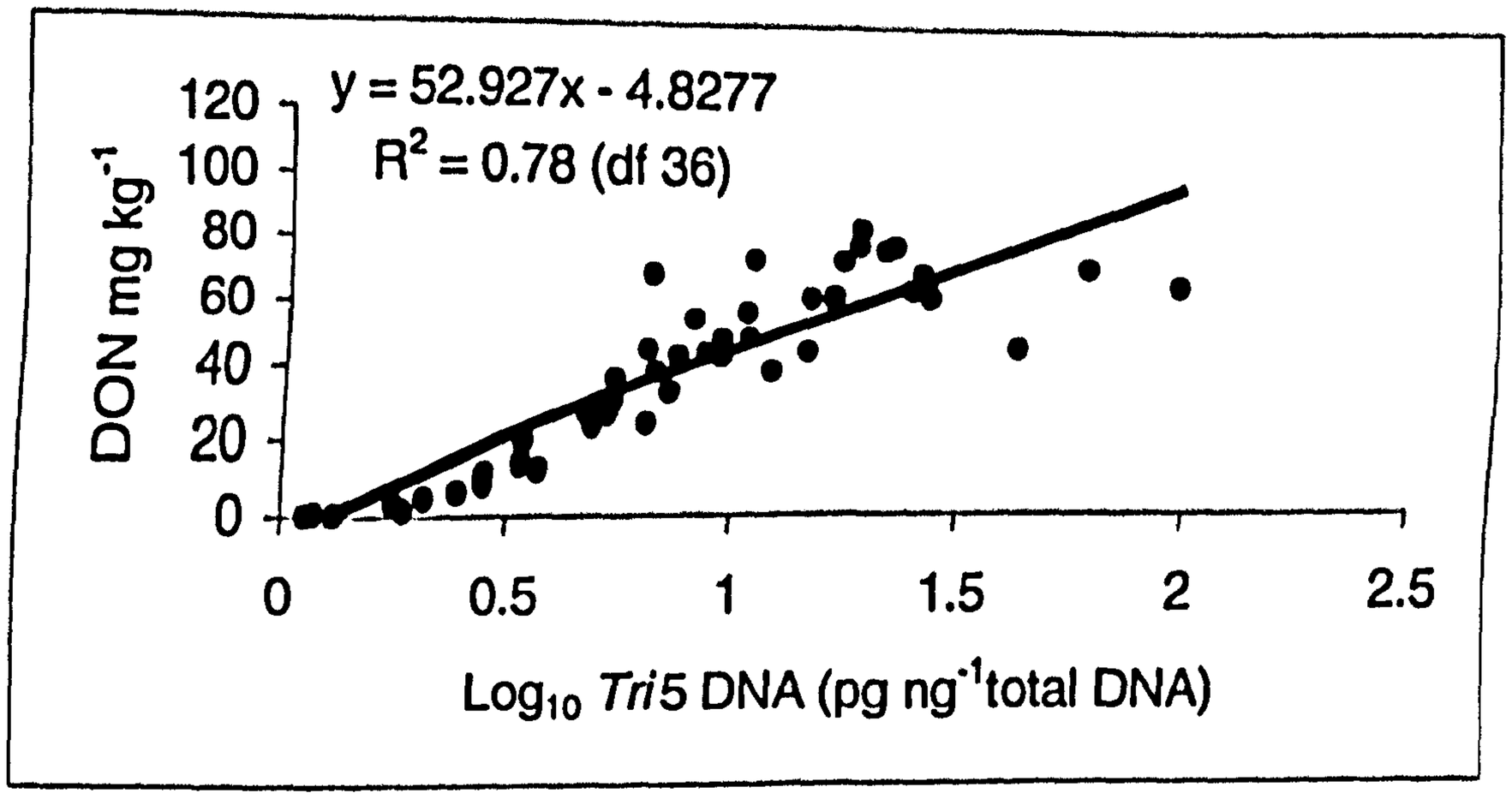


Figure 5.2 Relationship between quantity of *Tri*5 DNA and DON concentration in grain of winter wheat (cultivar Cadenza) in glasshouse experiment 2.

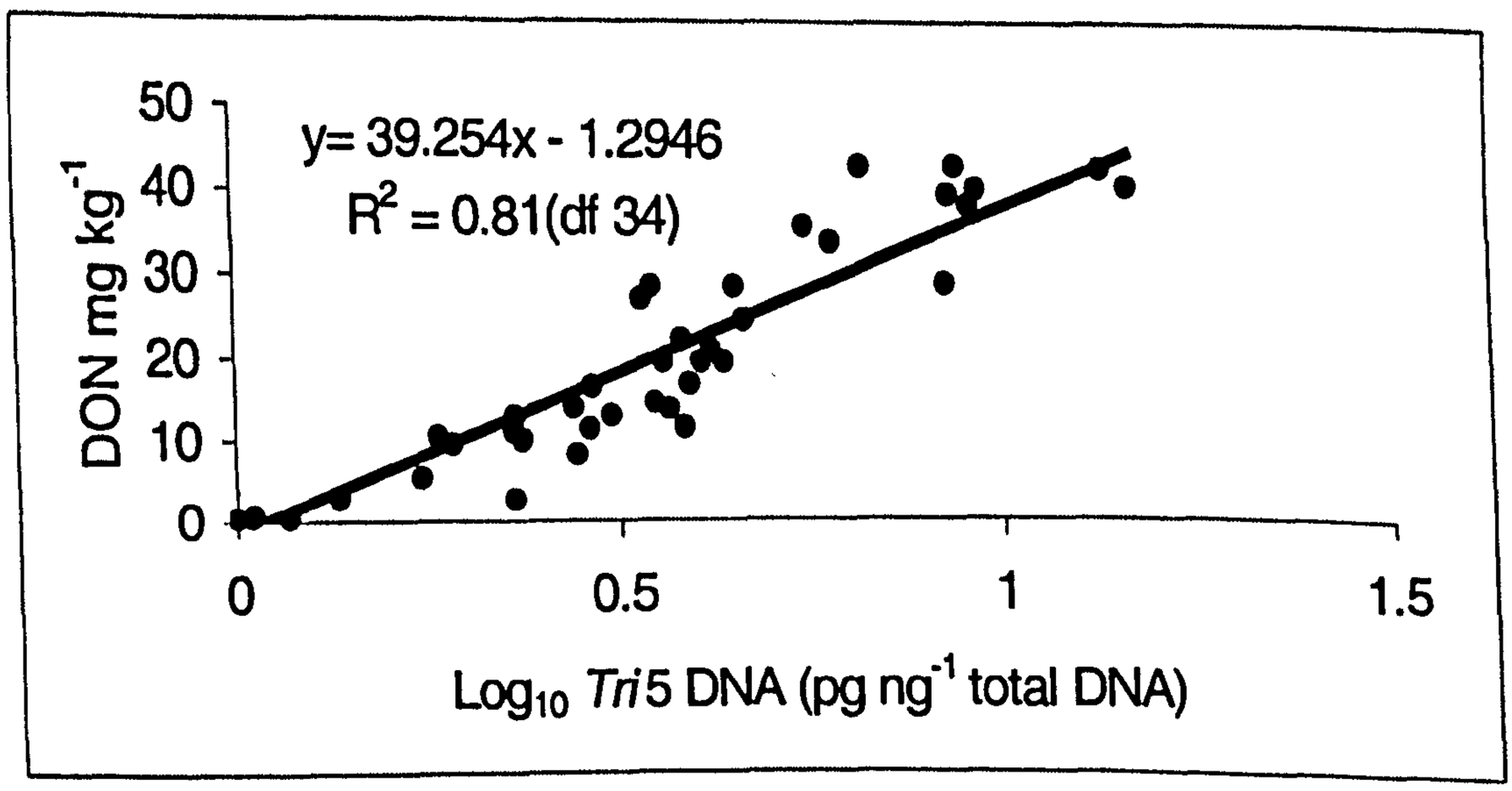


Figure 5.3 Relationship between quantity of *Tri*5 DNA and DON concentration in grain of winter wheat (cultivar Cadenza) in glasshouse experiment 3.

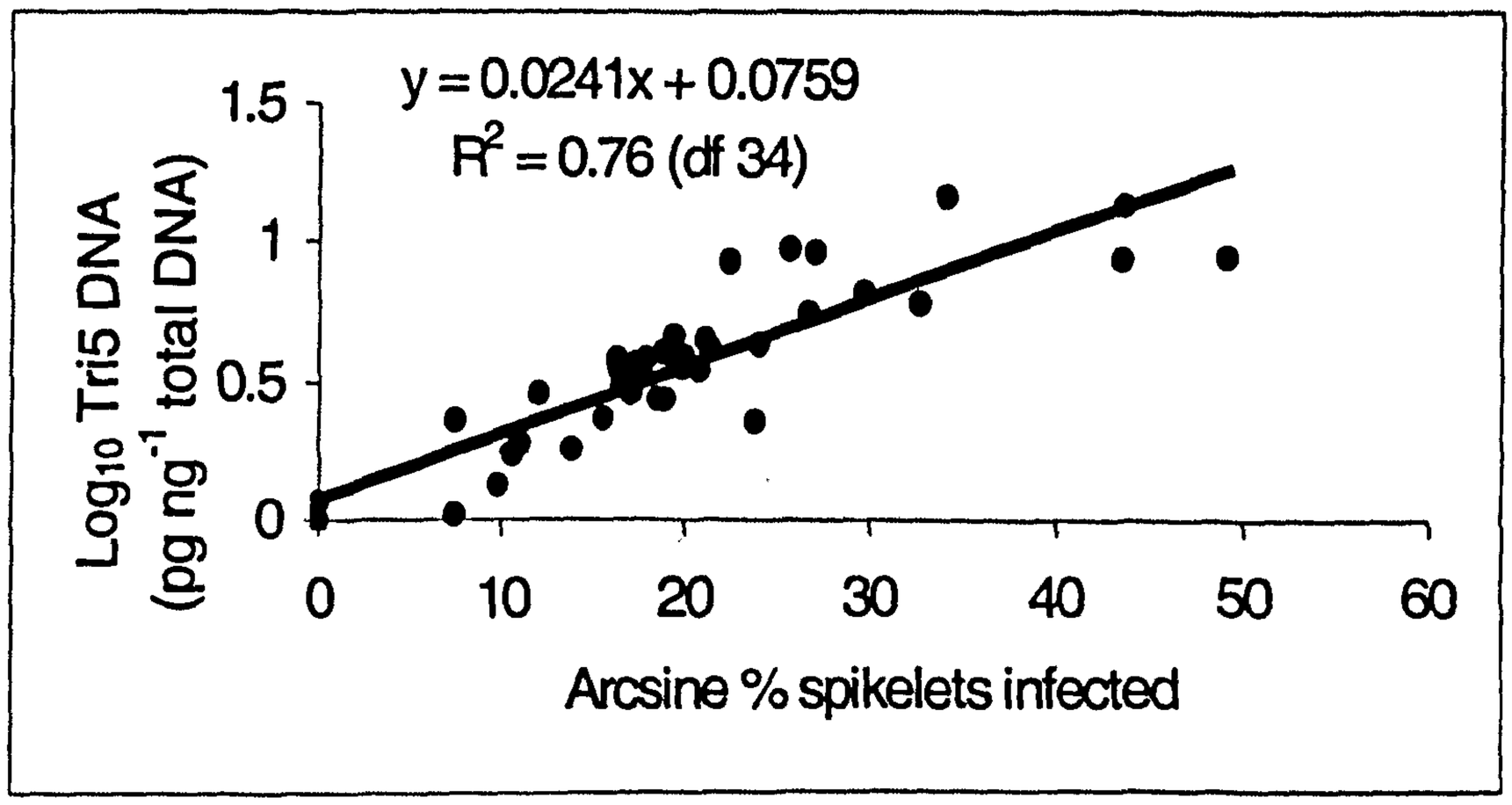


Figure 5.4 Relationship between severity of FHB (arcsine % spikelets infected) of winter wheat (cultivar Cadenza) and quantity of *Tri5* DNA in glasshouse experiment 3.

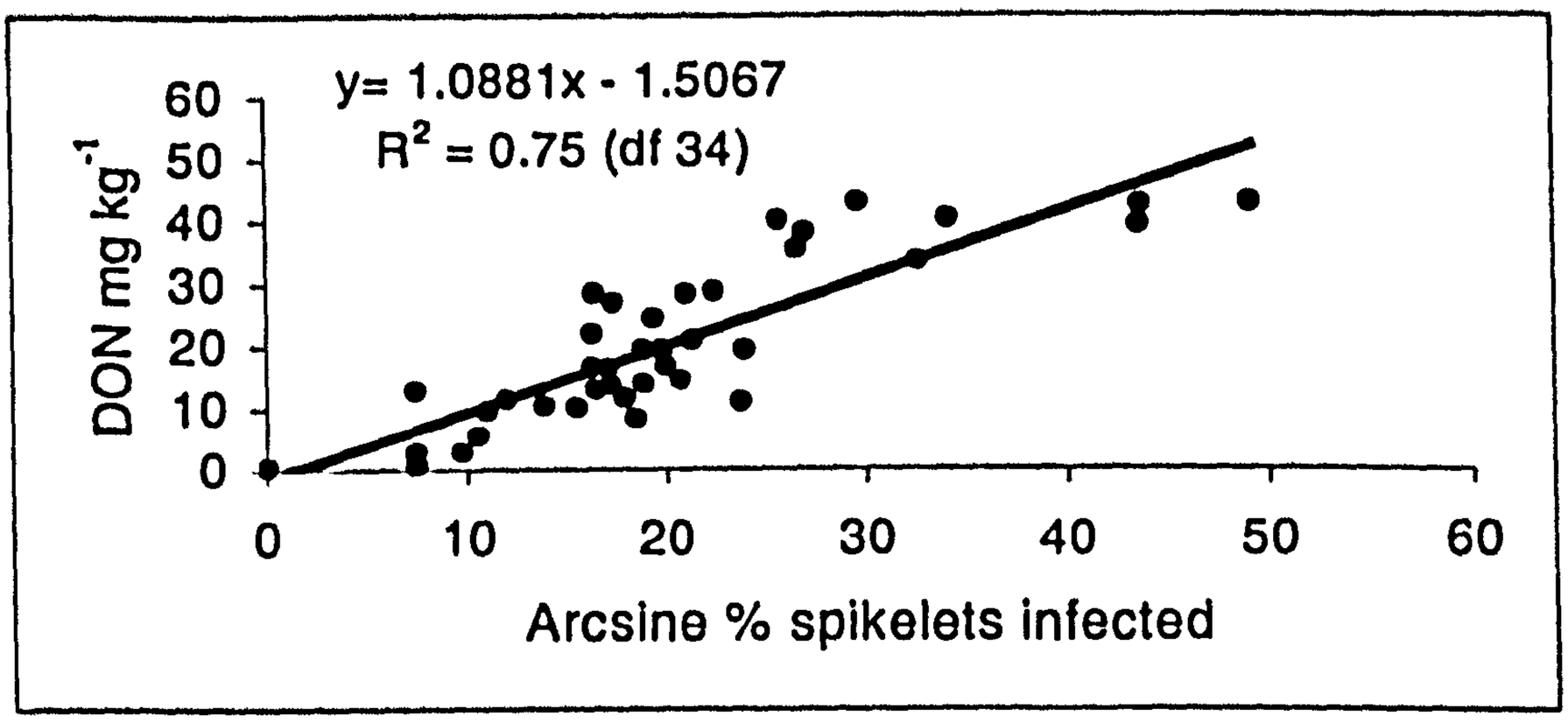


Figure 5.5 Relationship between the severity of FHB (arcsine % spikelets infected) of winter wheat (cultivar Cadenza) and DON content in grain in glasshouse experiment 3.

5.4 Discussion

The inoculation of wheat plants with *A. tenuissima* before or after the introduction of *F. culmorum* had no significant effect on FHB development, but resulted in an increase of *Tri5* DNA in harvested grain. However, this increase in *Tri5* DNA in grain did not result in a significant increase in DON concentration when compared to the control treatment. This is contrary to the work of Liggitt *et al.* (1997) who found a significant reduction (by 78%) of FHB symptoms when *A. alternata* was introduced to wheat ears at GS 59, before the introduction of *F. culmorum* at GS 65. Similarly, *in vitro* work by Liggitt *et al.* (1997) showed that when *F. culmorum* was introduced onto PDA, four days after the introduction of *A. alternata* on the agar plate, a 44% reduction in mycelial growth of *F. culmorum* was observed when compared to the growth of *F. culmorum* in the absence of *A. alternata*. In contrast, Magan and Lacey (1984) showed that when grown on malt agar at 25°C and 0.98 water activity, *F. culmorum* inhibited the growth of *C. herbarum* but did not inhibit the growth of *A. alternata in vitro*. However, when incubated under conditions of 25°C and 0.95 water activity, *F. culmorum* significantly inhibited the growth of both *A. alternata* and *C. herbarum*. When the same species have interacted on wheat extract agar under the same conditions, *C. herbarum* was only inhibited by *F. culmorum* at 0.95 water activity, whilst at 0.98 water activity *F. culmorum* was uncompetitive and intermingled freely with both *C. herbarum* and *A. alternata*.

In contrast to these observations, results recorded in these experiments showed that *A. tenuissima* and *C. herbarum* increased FHB severity by 56 and 60%, respectively, when introduced onto wheat ears at GS 57 before *F. culmorum*, and reduced disease symptoms by 40 and 50%, respectively, when introduced 24 hours after the introduction of *F. culmorum*. Liggitt *et al.* (1997) who reported a 63% reduction of FHB severity when *C. herbarum* was introduced onto wheat ears at GS 59, before the introduction of *F. culmorum* at GS65, observed no significant effect on disease severity when this fungus was introduced after *F. culmorum* at GS 69. However, Liggitt (1997) did observe an increase in FHB severity by 177% when *C. herbarum* was introduced to wheat ears at GS 57 before the introduction of *M. nivale* at GS 65, and by 139% when *C. herbarum* was introduced to ears at GS 69 after inoculation with *M. nivale*. It could be suggested, therefore, that the different pathogens involved in the FHB complex may react differently to the presence of different

saprophytic species. Another possible reason for the discrepancy between data obtained from these studies and these of Liggitt *et al.* (1997) could be due to the fact that Liggitt *et al.* (1997) used 200000 spores per ml of water for plant inoculation, whilst in this study, the inoculum concentrations of the species involved were 150000 spores per ml of water. Further study is necessary to investigate whether different spore loads of saprophytic species could effect FHB development or mycotoxin concentration in grain.

The introduction of *C. herbarum* at GS 57 resulted in a significant increase in DON concentration in grain. This might either suggest possible competition between *F. culmorum* and *C. herbarum*, or that the presence of *C. herbarum* on wheat ears before *F. culmorum* might predispose plants to an attack by *F. culmorum*. However, further study is necessary to provide more information about FHB pathogens and saprophytic species.

Azoxystrobin has been shown by Bertelsen *et al.* (2001) to suppress *C. herbarum* growth on wheat leaves. The application of azoxystrobin before or after introduction of *C. herbarum* to wheat plants did not result in an increase in FHB symptoms or in DON concentration in grain when compared to the control treatment. However it resulted in a decrease when it was compared to the treatment when *C. herbarum* or *M. nivale* was introduced on wheat ears at GS 57 alone before *F. culmorum*. These results agree with the suggestion by Liggitt *et al.* (1997) that applications of certain fungicides may affect the interactions within the fungal communities due to their differential activity towards individual species.

One possible reason for the discrepancy between the results from these studies on the interaction between the saprophytic species and *F. culmorum* to those of Magan and Lacey (1984) and Liggitt *et al.* (1997), could be that *A. tenuissima* was used, a species which may be less competitive with *Fusarium* spp. than *A. alternata*. Furthermore, such discrepancies may be explained by different environmental conditions experienced during the separate studies. Additional studies are, therefore, required in order to determine the interaction between *Fusarium* spp. and saprophytic species under a range of temperature and humidity conditions.

Molecular studies by Doohan *et al.* (1998) on the FHB complex found that wheat ear samples taken from a field trial where plots had been artificially inoculated with *F. culmorum,* found that both *F. culmorum* and *M. nivale* were both frequently isolated from the same ear. Furthermore, they also observed that as the quantity of *F. culmorum* colonising individual ears increased, the quantity of *M. nivale* decreased and *vice versa.* As a result of such observations, these workers concluded that an antagonistic relationship existed between the two pathogens. Results from this study support the hypothesis that antagonism may exist between *F. culmorum* and *M. nivale* since the introduction of *M. nivale* prior to the artificial inoculation of ears with *F. culmorum* increased FHB by 265%. Furthermore, although *Tri5* DNA content in grain was unaffected, DON content was increased by 151%. It could be suggested that due to competition with *M. nivale*, *F. culmorum* produces more DON. DON production by *Fusarium* spp. has been shown to play an important role in the virulence of these species (Bai *et al.*, 2001). However, the possibility of *M. nivale* predisposing ears to greater infection by *F. culmorum* cannot be overlooked. Sturz and Johnston (1983) have previously reported that *F. poae* colonised barley ears early in the growing season predisposed them to greater infection by *F. graminearum* and *F. culmorum* later in the season. Regression analysis revealed a strong and significant relationship between *Tri5* DNA and DON concentration in grain (r^2=55, r^2=78, r^2= 0.81, P<0.001) (Figure 5.1, 5.2 and 5.3) in experiments 2 and 3 and do not indicate a cluster of points above the fitted line. This, therefore, suggest that the presence of *M. nivale* or *C. herbarum* and *A. tenuissima* do not increase DON production per copy of *Tri5* DNA and hence more DON is produced because of greater quantity of toxin-producing species colonising wheat ears. This, therefore, supports the theory of predisposition of greater colonisation by *F. culmorum* when *M. nivale* is already present rather than increased production of DON by *F. culmorum* to outcompete *M. nivale.*

Jennings *et al.* (2000) associated applications of azoxystrobin with an increased in DON concentrations in grain due to elimination of the competition between *F. culmorum* and M. *nivale* on wheat ears. In two years of field studies, application of tebuconazole, metconazole or carbendazim gave significant reductions of *Fusarium* spp. in grain and corresponding decrease in

DON concentration in grain. Application of these fungicides also resulted in an increase of *M. nivale* in grain. In the first year, azoxystrobin effectively controlled *M. nivale* which reduced the competition with *Fusarium* species and resulted in an increase in DON in grain by about 56%. In the second year *M. nivale* was not present on the wheat ears and no significant increase of DON in grain after treatment of azoxystrobin was detected.

These studies have clearly shown an increase in FHB development and DON concentration in grain when azoxystrobin was applied after the introduction of *M. nivale* to wheat ears at GS 57. Although this increase was not significantly different when compared to the control treatment, it could be suggested that under field conditions this could have a significant effect on FHB severity and DON in grain.

Chapter 6

General Discussion

6.1 General Discussion

Fusarium head blight (FHB) also known as scab, is a devastating disease in small grain cereals throughout the world (Burgess *et al.*, 1987; Parry *et al.*, 1995; McMullen *et al.*, 1997; Gilbert and Tekauz, 2000; Moschini *et al.*, 2001; Zhuping, 1994; Cromey *et al.*, 2001). The disease is caused by five major species *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *Microdochium nivale* and reduces yield by between 10 and 70% (Martin and Johnston, 1982; Windels, 2000; Moschini *et al.*, 2001), normally associated with a decrease of 1000-grain weight, specific grain weight and grain number per head (Chelkowski *et al.*, 2000; Golinski *et al.*, 2002).

Apart from reducing yield the most serious threat of FHB is the ability of several *Fusarium* spp. to produce a range of toxic metabolites (Krska *et al.*, 2001; Hussein and Brasel, 2001), which can cause a wide range of adverse toxicoses in animals and humans as well as other health disorders (Bath *et al.*, 1989; Rotter *et al.*, 1995; Li *et al.*, 1999; Li *et al.*, 2002).

Although cultural practices (Dill-Macky and Jones, 2000; Yi *et al.*, 2001), the application of biocontrol agents (Khan *et al.*, 1999; Bujold e*t al.*, 2001) and the exploitation of more resistant varieties (Buerstmayr *et al.*, 1999; Hilton *et al.*, 1999; Bai *et al.*, 2000) have been shown to reduce FHB, control of the disease and its associated mycotoxins has been heavily reliant on fungicide applications.

Fungicidal control of FHB has proved inconsistent with several reports highlighting successful control of FHB by fungicides (Mesterhazy and Bartók, 1996; Ellner, 1997; Jones, 2000, Cromey *et al.* 2001) whilst others report only partial or no control of the disease (Martin and Johnston, 1982; Milus and Parsons, 1994). Furthermore, several reports have suggested that the application of certain fungicides for the control of FHB can result in elevated concentrations of trichothecene mycotoxins in harvested grain (Gareis and Ceynova, 1994; Matthies *et al.*, 1999; Jennings *et al.*, 2000; Simpson *et al.*, 2001).

Such discrepancies between observations recorded following fungicide applications may be due to a number of reasons. For example, the choice of fungicide/mixture and its time of application may significantly influence the results obtained along with the fungicide dose rate used. Furthermore, the effect of the resistance of the variety used and the potential interaction between FHB-causing pathogens and other fungal species colonising wheat ears cannot be overlooked.

As a result of field trial 1(1998/99) presented in Chapter 3, it was shown that a mixture of metconazole+azoxystrobin applied at GS 59 was the most effective treatment reducing FHB severity by 63%, followed by applications of tebuconazole or metconazole alone. Azoxystrobin proved the least effective at controlling FHB. These observations may be explained by the fact that wheat plots were artificially inoculated with a mixture of the FHB pathogens *F. culmorum, F. graminearum* and *M. nivale*. In field trials carried out by Simpson *et al.* (2001), applications of tebuconazole where shown to reduce significantly ($P<0.001$) the amount of *Fusarium* spp. in harvested grain but had no significant effect on levels of *M. nivale* ($P>0.2$). In contrast, fungicide treatments containing azoxystrobin effectively controlled *M. nivale* ($P<0.01$) but did not significantly reduce levels of *Fusarium* species ($P>0.2$). This was supported by the results from plating out of grain onto PDA in this trial where the presence of *M. nivale* was significantly ($P<0.001$) affected by the application of azoxystrobin at both rates. Results from the first field trial are also in agreement with the work of Jones (2000) who reported that applications of tebuconazole and propiconazole against FHB (*F. graminearum*) in barley, significantly reduced FHB severity by 39% and 31%, whilst azoxystrobin was the least effective treatment.

The quantification of *Tri5* DNA in grain and DON concentration in grain showed that metconazole and tebuconazole applied at full rate provided effective control of trichothecene-producing *Fusarium* spp. and reduced *Tri5* DNA in harvested grain by 84 and 75%, respectively. Metconazole and tebuconazole were also shown to significantly reduce the concentration of DON in harvested grain by 82% and 83%, respectively. Azoxystrobin applied at both rates had no significant effects on either *Tri5* DNA quantity in grain or DON concentration ($P<0.05$). These

results are contrary to data obtained from several *in vitro* and *in vivo* studies. For example, *in vitro* work by Matthies *et al.* (1999) showed that although tebuconazole at sublethal concentrations significantly ($P<0.05$) reduced mycelial growth of *F. graminearum* on potato dextrose broth and enhanced 3-ADON concentration four times in comparison with control. Thiabendazole also reduced fungal growth but 3-ADON concentration considerably increased compared to the fungicide-free control. Milus and Parsons (1994) studied the effect of benomyl, chlorothalonil, fenbuconazole, flusilazole, myclobutanil, potassium bicarbonate, propiconazole, tebuconazole, thiabendazole and triadimefon plus mancozeb against FHB severity, DON contamination and yield of winter wheat. During two years of field studies, these authors did not observed any significant effect of these fungicides on either FHB or DON levels in harvested grain.

The timing of fungicide application appears to be an important factor in effectively suppressing FHB with fungicides. In field trial 2(1999/2000) (Chapter 3) where fungicides were applied early in the growing season (GS 31+39), no effect on FHB severity was recorded at GS 85, whilst metconazole+azoxystrobin applied at GS 39+59 was the most effective treatment at reducing visual symptoms of FHB, followed by metconazole or azoxystrobin alone. The lack of any significant difference in FHB severity at GS 85 between metconazole and azoxystrobin was probably due to the high level of *M. nivale* natural infection of wheat ears and that while metconazole reduced the amount of *Fusarium* spp., symptom development was masked by *M. nivale*, This suggestion is supported by the quantification of *Tri*5 DNA and *M. nivale* DNA in harvested grain, which clearly indicated that metconazole was effective at reducing grain infection by *Fusarium* spp. whilst azoxystrobin reduced the infection by *M. nivale*. The application of metconazole at GS 39+59 provided significant reduction of % FDK that agrees with observations recorded by Dardis and Walsh (2000). The application of metconazole at either GS 31+39 or GS 39+59, did not provide any significant ($P>0.05$) reduction of DON in harvested grain which contrasts with the results obtained from field trial 1(1998/1999).

Such a discrepancy observed in field trial 2(1999/2000) might be due to an excessively high disease pressure induced by the high amount of inoculum placed into the field early in the growing

season. Milus and Parsons (1994) reported no effect of a range of fungicides including tebuconazole against FHB severity and DON concentration in grain and attributed this to the high disease pressure during the two years of investigation. This is also supported by the fact that fungicides had no significant effect ($P>0.05$) on the incidence of *Fusarium* spp. and *M. nivale* on the wheat leaves at any of the sampling stages which suggests that even if fungicide treatments have provided a significant effect of the incidence of FHB pathogens on the wheat leaves immediately after their application, within two to three days after, the wheat leaves may have been re-infected by fungal propagules dispersed during subsequent rainfall events. Based on these results, it could be concluded that the application of fungicides early in the growing season (GS 31-39) could have some effect on the incidence of *Fusarium* spp. on the leaves and stems of wheat plants but not on the severity of FHB, grain colonisation by *Fusarium* spp. and DON concentration in grain. However, this study also showed that in order to achieve better control of FHB, investigations on the timing of fungicides against the disease should be focused around heading (GS 59-67).

In field trial 3(2000/2001) (Chapter 3), factorial ANOVA revealed that all fungicides provided significant control disease severity at GS 85 when compared with untreated plots ($P<0.001$). The fungicide treatments were more effective against FHB when they were applied 2 days before or 2 days after inoculation of the wheat plots at GS 65. This is in agreement with the work by Mauler-Machnik and Zahn (1994) who applied tebuconazole either 3 days before or 3 days after the artificial inoculation of wheat ears with *Fusarium* spp. at GS 65 resulted in a 60% reduction in FHB severity when compared to untreated controls. All triazole fungicides significantly decreased DON concentration in grain when applied 5 days after inoculation but were less effective in comparison with those applied 2 days before or 2 days after inoculation. Similar effects have been observed by Homdork *et al.* (2000) who found that applications of tebuconazole against FHB caused by *F. culmorum* reduced DON in grain by 69% when applied 3 days before inoculation, whilst an application 5 days post-inoculation reduced DON by 54%. Siranidou and Buchenauer (2001) also demonstrated that tebuconazole applied 2 days before or two days after inoculation of wheat plots inoculated with *F. culmorum* resulted in a reduction of DON by 71 and 62%, respectively. This

suggests that fungicide application is more effective in reducing FHB and DON when treatments are applied closer to the beginning of fungal infection, before disease is well established.

With the exception of field trial 2(1999/2000) (Chapter 3), triazole fungicides provided significant control of FHB severity, *Tri*5 DNA quantity and DON concentration in grain. These findings are supported by those recorded in Chapter 4 where metconazole applied at all four rates (quarter, half, full and double the manufacturer's recommended dose rate) against FHB caused by *F. culmorum* or *F. graminearum* resulted in a significant reduction of disease symptom development, reduction of trichothecene-producing species in grain and reduction of DON concentration in grain. Application of azoxystrobin against FHB has been reported to increase DON concentration in grain. For example, Jennings *et al.* (2000) attributed application of azoxystrobin to significant increases in DON production in comparison to control treatment and suggested that applications of azoxystrobin reduced *M. nivale* on wheat ears and altered the proportion of trichothecene-producing *Fusarium* within the complex which in turn resulted in an increase in mycotoxin production. More recently, Simpson *et al.* (2001) have also associated applications of azoxystrobin with increased DON concentrations in harvested wheat grain. During a field trial where wheat ears were artificially inoculated with a mixture of *F. avenaceum*, *F. culmorum* and *M. nivale* at GS 65 before being sprayed with azoxystrobin three days later, these workers observed a 40% increase in DON concentration in harvested grain when compared to grain harvested from unsprayed plots. The quantification of *F. culmorum* DNA did not indicate an increase of this species in grain, although *M. nivale* DNA was significantly reduced by azoxystrobin. This result could indicate that applications of the fungicide azoxystrobin might have a direct effect on DON production. In contrast with these findings, none of the azoxystrobin applications during the three years of field studies (Chapter 3) or the two glasshouse experiments in Chapter 4 in this project showed any significant detrimental effect of that particular fungicide on FHB severity, *Tri*5 DNA and DON concentration in grain even when azoxystrobin was applied at double the normal rate.

Results from the field studies in Chapter 3 and the glasshouse experiments in Chapter 4 indicate that DON concentration present in harvested grain was strongly influenced by the concentration of the

Tri5 gene in grain as determined by the competitive PCR assay. The regression analysis revealed strong relationships between *Tri5* DNA and DON concentration in grain (Figure 3.2 a, b and 4.1 a, b). Such strong relationships between *Tri5* and DON concentration suggest that in this study, the fungicide treatments tested did not elevate DON concentration over and above that which was determined by the quantity of trichothecene-producing *Fusarium* present in grain. Had an individual treatment resulted in a cluster of points above the regression line, this would have suggested that such a treatment increased the concentration of DON produced per *Tri5* copy. The lack of any obvious cluster indicates that neither azoxystrobin, the triazole fungicides used in these studies or their mixtures with azoxystrobin influenced DON concentration within grain other than by altering the amount of trichothecene-producing *Fusarium* present.

All fungicide treatments had a significant but inconsistent effect on yield of winter wheat during the three field trials. One possible reason for these variable effects of fungicides on yield may be the differential control of foliar pathogens. The use of harvesting machinery, which may have allowed small and shrivelled grain to be lost during harvest could have resulted in small differences within grain parameters among fungicide treatments.

The experimental studies on the effect of fungicides on the interaction between *F. culmorum* and *A. tenuissima*, *C. herbarum* and *M. nivale* (Chapter 5) showed that when these species were present on wheat plants at GS 57 before introduction of *F. culmorum* on wheat ears at GS 65, with the exception of *A. tenuissima*, there was a significant increase of FHB severity, *Tri5* DNA and DON concentration in grain. Although applications of azoxystrobin did not result in a significant increase of FHB symptoms, *Tri5* DNA or DON in grain compared to the control treatment when this was applied after or before inoculation of wheat plants with either of the saprophytic species, an increase of DON by 56% and 30% was observed when this fungicide was applied after or before introduction of M. *nivale* on wheat plants respectively. This data supports findings by Jennings *et al.* (2000) who explained significant increases in DON production in comparison to control treatment with the application of azoxystrobin against FHB and suggested that applications of azoxystrobin reduced *M. nivale* on wheat ears and altered the proportion of trichothecene-producing *Fusarium* within the

complex which in turn resulted in an increase in mycotoxin production. However, the addition of *M. nivale* at GS 57 before *F. culmorum* at GS 65 in this study lead to a significant increase of DON concentration. The application of azoxystrobin under these conditions resulted in a significant decrease of DON in grain by 47%. This would suggest that the early presence of *M. nivale* on wheat ears results in predisposition to *F. culmorum* attack and the application of azoxystrobin disrupts the possible predisposition. This also shows that the time of fungicide application play an important role in the fungicidal control of FHB.

Due to the complex nature of the interactions between *Fusarium* spp. and other species occurring on wheat ears under field conditions, coupled with the application of fungicides it is difficult to draw clear conclusions. It would not be spurious to suggest that poor performance of fungicides under field conditions and increases of mycotoxin concentrations in grain of wheat after application of particular fungicides such as azoxystrobin, could be due to the presence of non-target species such as *Alternaria* spp. or *Cladosporium* spp. or non-toxin producing species such as *M. nivale.* For example, glasshouse studies by Liggitt *et al.* (1997) demonstrated that wheat plants inoculated with either, *A. alternata*, *B. cinerea* or *C. herbarum* at GS 59 prior to inoculation with *F. culmorum* at GS 65 resulted in a decrease of FHB severity between 46% and 78% compared to plants inoculated only with *F. culmorum.* In an investigation on the interaction between *Bipolaris sorokiniana* and *F. graminearum* on the ears of barley, Tekauz and McCallum (2000a) found a 91% reduction in FHB symptoms when *B. sorokiniana* was present on barley ears before the introduction of *F. graminearum.* There are two possible ways, which could lead to an increase of mycotoxin concentration in grain as a result of these interactions. Firstly fungicides with limited activity against *Fusarium* spp. when applied to wheat could lead to greater colonisation of wheat by the pathogen, due to the removal of antagonistic saprophytes, and to the consequent increase of mycotoxin concentration in grain. Secondly, fungicides applications may disrupt predisposition to the FHB pathogens.

6.2 Proposed further studies

In this study, the effectiveness of fungicides against FHB and mycotoxin accumulation in wheat has been demonstrated. The results have clearly shown that the fungicides have no direct effect on mycotoxin concentration in grain. Furthermore the results have also shown that performance against FHB is influenced by the presence of fungal species other than FHB causing pathogens. In order to further our understanding of factors which influence the efficacy of fungicides against FHB and mycotoxin accumulation, the following areas of research are recommended:

- Further controlled environment studies to determine the interaction between *M. nivale* and *C. herbarum* with *F. culmorum* with regard to FHB development and mycotoxin production. Such studies would involve studying the interactions between these fungi at temperatures ranging from 10-20°C and Relative Humidity ranging between 90-98%.

- Data from Chapter 5 suggests that M. *nivale* may predispose wheat ears to greater colonisation by *Fusarium* spp. Therefore, further ultrastructural and cytochemical studies are necessary (Kang and Buchenauer, 2000; Kang and Buchenauer 2002) to investigate the changes in wheat spikes that may occur when *M. nivale* is inoculated onto wheat ears at GS 57, 60 or 69 and how this may affect subsequent *F. culmorum* invasion.

- Liggitt *et al.* (1997) reported a decrease in FHB development when the saprophytic species (*A. alternata*, *C. herbarum* or *B. cinerea* (at rate 250000 spores per ml of water) were introduced to wheat ears before inoculation with *F. culmorum*. In Chapter 5 of this study when lower inoculum concentrations (150000 spores per ml of water) of *C. herbarum* and *M. nivale* were introduced to wheat ears before *F. culmorum*, a significant increase of FHB was observed. Further studies are necessary to investigate the effect of different spore loads (25000; 50000; 100000; 200000 and 400000 spores per ml of water) of these species on FHB development.

- Further studies to determine the interaction between different *Fusarium* spp. and the effect on FHB development and mycotoxin production. For example: wheat plants should be inoculated with *F. culmorum* alone (control) and in combination with other FHB causing pathogens: *F. culmorum* + *F. avenaceum*; *F. culmorum* + *F. graminearum*; *F. culmorum* + *F. poae*; *F. culmorum* + *F. avenaceum* + *F. graminearum*; *F. culmorum* + *F. avenaceum* + *F. poae*; *F. culmorum* + *F. graminearum* + *F. poae*. *M. nivale* could also be added to these combinations.

- Results from Chapter 4 suggests that fungicide efficacy against FHB can be significantly affected by prevailing environmental conditions. Further controlled environment studies are recommended to determine the effect of a range of temperature (15°C, 20°C, 25°C, 30°C) and relative humidity (90%, 95% and 98%) regimes on fungicide performance against FHB and the production of DON and other *Fusarium* toxins (NIV, DON and DON acetilated derivatives).

- Work by Mesterhazy and Bartók (1996) showed that a combination of effective fungicides and more resistant wheat varieties could provide better control of FHB. Further field studies to determine the efficacy of fungicides and dose rates on a number of wheat cultivars which differ in resistance to FHB mycotoxin accumulation should be carried out.

Bibliography

Adler von A, Lew H, Edinger W, 1990. Incidence and toxigenicity of *Fusarium* species in cereals from Austria. *Bodenkultur* **41**, 145-152.

Andersen AL, 1948. The development of *Gibberella zeae* head blight of wheat. *Phytopathology* **38**, 595-611.

Aoki T and O'Donnell K, 1999. Morphological and molecular characterisation of *Fusarium pseudograminearum* sp.nov. formerly recognised as the Group 1 population of *F. graminearum*. *Mycologia* **91**, 957-609.

Apony I, Nagy G, Princimger, G, Kajati I, 1998. Fusarium infection of wheat seeds in Hungary between 1970 and 1991. *Cereal Research Communications* **26**, 253-258.

Arthur JC, 1891. Wheat scab. Indiana Agricultural Experimental Station, *Bulletin* **36**, 129-132.

Arseniuk E, Góral T, Czembor HJ, 1993. Reaction of triticale, wheat and rye accessions to graminaceous *Fusarium* spp. infection at the seedling stage and adult plant growth stages. *Euphytica* **70**, 175-183.

Arseniuk E, Foremska E, Góral T, Chelkowski J, 1999. Fusarium head blight reactions and accumulation of deoxynivalenol (DON) and some of its derivatives in kernels of wheat, triticale and rye. *Journal of Phytopathology* **147**, 577-590.

Atanasoff D, 1920. Fusarium blight (scab) of wheat and other cereals. *Journal of Agricultural Research* **20**, 1-31.

Bai GH, Shaner G, Ohm H, 2000. Inheritance of resistance to *Fusarium graminearum* in wheat. *Theoretical and Applied Genetics* **100**, 1-8.

Bai GH, Desjardins AE, Plattner RD, 2001. Deoxynivalenol-nonproducing *Fusarium graminearum* causes initial infection, but does not cause disease spread in wheat spikes. *Mycopathologia* **153**, 91-98.

Bakan B, Cahagnier B, Melcion D, 1998. Natural occurrence of *Fusarium* toxins in domestic wheat and corn harvested in 1996 and 1997 and production of mycotoxin by *Fusarium* isolates from these sample. *Revue de Mèdecine Vètèrinarie* **6**, 697.

Balmas V, Maccaroni M, Santori, Corazza L, 1999. Fusariosi della spiga di cereali autonnovernini: indagine epidemiologica e valutazione della resistenza di frumento duro nell'Italia centrale. *Informatore fitopatologico* **12**, 37-42.

Bateman GL, 1979. Relationship between *Fusarium nivale* and other micro-organisms on seed of wheat and barley. *Transactions of the British Mycological Society* **72**, 245-249.

Bateman GL, 1983. Response of *Fusarium nivale* in wheat and barley caryopses to organomercury treatment in relation to site of infection and microbial antagonism. *Transactions of the British Mycological Society* **81**, 141-183.

Bechtel DB, Kaleikau LA, Gaines RL, Seitz LM, 1985. The effects of *Fusarium graminearum* infection on wheat kernels. *Cereal Chemistry* **62**, 191-197.

Bertelsen JR, de Neergaard E, Smedegaard-Petersen, V 2001. Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. *Plant Pathology* **50**, 190-205.

Bhat RV, Ramakrishna Y, Beedu SR, Munshi KL, 1989. Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat products in Kashmir valley India. *The Lancet* **7**, 35-37.

Birzele B, Prange A, Krämer J, 2000. Deoxynivalenol and ochratoxin A in German wheat and changes of level in realtion to storage parameters. *Food Additives and Contaminants* **17**, 1027-1037.

Blaney BJ, Moore CJ, Tyler AL, 1987. The Mycotoxins-4-deoxynivalenol, zearalenone and aflatoxin-in weather-damaged wheat harvested 1983-1985 in south-eatsern Queensland. *Australian Journal of Agricultural Research* **38**, 993-1000.

Boosalis M, Doupnik B, Wysong D, Watkins J, 1983. The wheat scab epidemic of 1982. *Nebraska Farm, Ranch and Home Quarterly* **29**, 7-9.

Booth C 1971. The genus *Fusarium*. Commonwealth Mycological Institute.

Bottalico A and Logrieco A, 2001. Distribution of toxigenic *Fusarium* species and mycotoxins associated with head blight of wheat, in Europe. In: Proccedings of Sustainable Systems of Cereal Crop Protection against Fungal diseases as the Way of Reduction of Toxin Occurrence in Food Webs, 2-6 July, Kroměříž, Czech Republic, pp.83-88.

Boyacioglu D, Hettiarachchy NS, Stack RW, 1992. Effect of three systemic fungicides on deoxynivalenol (vomitoxin) production by *Fusarium graminearum* in wheat. *Canadian Journal of Plant Science* **72**, 93-101.

Bryden WL, Logrieco A, Abbas HK, Porter JK, Vesonder RF, Richard JL and Cole RJ, 2001. Other significant *Fusarium* mycotoxins 361-392. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL and Burgess LW (Eds.) *Fusarium*-Paul E. Nelson Memorial Symposium. APS press, St. Paul, Minnesota.

Buerstmayr H, Lemmens M, Berlakovich S, Ruckenbauer P, 1999. Combining ability of resistance to head blight caused by *Fusarium culmorum* (W.G. Smith) in the F_1 of a seven parent diallel of winter whet (*Triticum aestivum* L.). *Euphytica* **110**, 199-206.

Bujold I, Paulitz TC, Carisse, O, 2001. Effect of *Microsphaeropsis sp.* on the production of perithecia and ascospores of *Gibberella zeae*. *Plant Disease* **85**, 977-984.

Bullerman LB, 1979. Significance of mycotoxins to food safety and human health. *Journal of Food Protection* **42**, 65-68.

Burgess LW, Klein TA, Bryden WL, Tobin NF, 1987. Head blight of wheat caused by *Fusarium graminearum* Group 1 in NSW in 1983. *Australian Journal of Plant Pathology* **16**, 72-78.

Campbell H, Choo TH, Vigier B, Underhill L, 2000. Mycotoxins in barley and oat samples from eastern Canada. *Canadian Journal of Plant Science* **80**, 977-980.

Carranza MR, 1988. Chemical control of wheat spike blight. *Annals of Applied Biology* **112**, 38-39.

Cassini R, 1981. Fusarium disease of cereals in Western Europe. In: Nelson PE, Tussoun TA, Cook RJ (Eds.) Fusarium: Diseases, Bology and Taxonomy. University Park: Pennsylvania State University Press, pp.56-63.

Chaudhary RG, Edison S, Vishvadhar, 1990. Epidemiology and basic factors of severity, yield losses and grain quality deterioration due to ear blight of wheat in Arunachal Pradesh. *Indian Phytopathology* **43**, 571-574.

Chelkowski I, Tomczak M, Basinski T, Perkowski J, Kotecki M, Stachowiak J, Kaptur P, Gloiński PK, 2000. Contamination of cereals in Poland with *Fusarium* toxins. In: Proceedings of the 6th European Fusarium Seminar and Third COST 835 Workshop, 11th-16th September, Berlin, Germany, pp.111.

Clear RM, Patrick SK, Gaba D, 2000. Prevalence of fungi and fusariotoxins on barley seed from western Canada, 1995-1997. *Canadian Journal of Plant Pathology* **22**, 44-50.

Ĉote LM, Dahlem AM, Yoshizawa T, Swanson SP, Buck WB, 1986. Excretion of deoxynivalenol and its metabolite in milk, urine, and feces of lactating dairy cows. *Journal of Dairy Science* **69**, 2416-2423.

Couture L, Dubuc JP, Comeau A, 1995. Occurrence of contamination of wheat grain by the orange wheat midge and seed microflora in Québec. *Canadian Plant Disease Survey* **75**, 128-129.

Cromey MG, Lauren DR, Parkes RA, Sinclair KI, Shorter SC, Wallace AR, 2001. Control of Fusarium head blight of wheat with fungicides. *Australasian Plant Pathology* **31**, 301-308.

Curtui V, Usleber E, Dietrich R, Lepschy J, Märtlbauer, E 1998. A survey on the occurrence of mycotoxins in wheat and maize from western Romania. *Mycopathologia* **143**, 97-103.

Daamen RA, Langerak CJ, Stol W, 1991. Surveys of cereal diseases and pests in the Netherlands. 3. *Monographella nivalis* and *Fusarium* spp. in winter wheat fields and seed lots. *Neteherland Journal of Plant Pathology* **97**, 105-114.

Dardis J and Walsh EJ 2000. Studies of the effectiveness of metconazole in controlling Fusarium head blight caused by *Fusarium culmorum* in spring wheat (*Triticum aestivum* L.). *Cereal Research Communications* **28**, 443-448.

D'Mello JPF, MacDonald AMC, Postel D, Hunter EA, 1997. 3-Acetyl deoxynivalenol production in a strain of *Fusarium culmorum* insensitive to the fungicide difenconazole. *Mycotoxin Research* **13**, 73-79.

D'Mello JPF, MacDonald AMC, Briere L, 2000. Mycotoxin in a carbendazim-resistant strain of *Fusarium sporotrichioides*. *Mycotoxin Research* **16**, 101-111.

D'Mello JPF, MacDonald AMC, Rinna R, 2001. Effects of azoxystrobin on mycotoxin production in a carbendazim-resistant strain of *Fusarium sporotrichioides*. *Phytoparasitica* **29**, 431-440.

Dexter JE, Clear RM, Preston KR, 1996. Fusarium head blight: effect on the milling and baking of some Canadian wheats. *Cereal Chemistry* **73**, 695-701.

Dickson JG and Mains EB 1929. Scab of wheat and barley and its control. *US Department of Agriculture Farmers Bulletin* **1599**, 1-17.

Dickson JG, 1942. Scab of wheat and barley and its control. *US Department of Agriculture Farmers Bulletin* (revised) **1599**, 1-22.

Dill-Macky R and Jones RK 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. *Plant Disease* **84**, 71-76.

Doerr JA, Hamilton PB, Burmeister HR, 1981. T-2 toxicosis and blood coagulation in young chickens. *Toxicology and Applied Pharmacology* **60**, 157-162.

Doohan FM, Parry DW, Jenkinson P, Nicholson P, 1998. The use of species-specific PCR-based assays to analyse Fusarium ear blight of wheat. *Plant Pathology* **47**, 197-205.

Duben J and Fehrman H 1979. Occurrence and pathogenicity of *Fusarium* species on winter wheat in West Germany. II. Comparison of pathogenicity on seedlings, haulm bases and ears. *Journal of Plant Disease and Protection* **86**, 705-728.

Ellner FM, 1997. Influence of fungicide treatment on deoxynivalenol content in winter wheat artificially infected with *Fusarium culmorum*. *Cereal Research Communications* **25**, 735-737.

Ellner FM and Schroer R (2000) Effects of fungicides containing strobilurin on mycotoxin production in wheat. In: Proceedings of the 6th European Fusarium Seminar, 11-16 September 2000,Berlin, Germany, pp 101.

Evans CK, Xie W, Dill-Macky R, Mirocha, CJ 2000. Biosynthesis of deoxynivalenol in spikelets of barley inoculated with macroconidia of *Fusarium graminearum*. *Plant Disease* **84**, 654-660.

Fazekas B, Hajdu ET, Tar AK, Tanyi J, 2000. Natural deoxynivalenol (DON) contamination of wheat samples grown in 1998 as determined by high-performance liquid chromatography. *Acta Veterinaria Hungarica* **48**, 151-160.

Francis RG and Burges LW 1977. Characteristics of *Fusarium roseum* '*Graminearum*' in Eastern Australia. *Transactions of the British Mycological Society* **68**, 421-427.

Gagkaeva T and Levitin M 1997. Composition of *Fusarium graminearum* Schwabe populations collected from different regions of Russia. *Cereal Research Communications* **25**, 591-593.

Gareis M and Ceynova J 1994. Influence of the fungicide Matador (tebuconazole/triadimenol) on mycotoxin production by *Fusarium culmorum*. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung* **198**, 244-248.

Gerlach W and Nierenberg H 1982. The Genus Fusarium-a Pictorial Atlas. Berlin; Dahlem Heft 209, pp.406.

Gilbert J and Tekauz A 2000. Recent developments in research in fusarium head blight of wheat in Canada. *Canadian Journal of Pathology* **22**, 1-8.

Gilbert J, 2001. Effects of *Fusarium graminearum* infection of wheat seed. In: Proceedings of Sustainable Systems of Cereal Crop Protection against Fungal diseases as the Way of Reduction of Toxin Occurrence in Food Webs, 2-6 July, Kroměříž, Czech Republic, pp.35-40.

Glynn NC, Edwards SG, Hare MC, Burke R, Parry DW, 2000. Quantification of *Fusarium* spp. and *M. nivale* in fungicide seed treatment efficacy trials. In: Proceedings of 6th European Fusarium Seminar and Third COST 835 Workshop, 11-16 September, 2000, Berlin, Germany, pp.40-41.

Golinski P, Kaczmarek Z, Kiecana I, Wisniewska H, Kaptur P, Kostecki M, Chelkowski J, 2002. *Fusarium* head blight of common Polish winter wheat cultivars – comparison of effects of *Fusarium avenaceum* and *Fusarium culmorum* on yield components. *Journal of Phytopathology* **150**, 135-141.

Gordon WL, 1959. The occurrence of *Fusarium* species in Canada. VI. Taxonomy and geographical distribution of *Fusarium* species on plants, insects and fungi. *Canadian Journal of Botany* **37**, 257-290.

Hacking A, Rosser WR, Dervish MT, 1976. Zearalenone-producing species of *Fusarium* on barley seed. *Annals of applied Biology* **84**, 7-11.

Hamilton RMG, Trenholm HL, Thompson BK, Greenhalgh R, 1985a. The tolerance of white Leghorn and broiler chicks, and turkey poults to diets that contained deoxynivalenol (vomitoxin)-contaminated wheat. *Poultry Science* **64**, 273-286.

Hamilton RMG, Trenholm HL, Thompson BK, Fiser PS, Greenhalgh R, 1985b. Effects of feeding white Leghorn hens diets that contain deoxynivalenol (vomitoxin)-contaminated wheat. *Poultry Science* **64**, 1840-1852.

Hamilton RMG, Thompson BK, Trenholm HL, 1986. The effects of deoxynivalenol (vomitoxin) on dietary preference of white Leghorn hens. *Poultry Science* **65**, 288-293.

Hart P and Ward R (1997). Efficacy of fungicides on *Fusarium* head blight severity and levels of vomitoxin. In: Proceedings of National Fusarium Head Blight Forum, 10-12 Nov. 1997, St.Paul, Minn, pp 40-41.

Hasan AHH, 1993. Fungicide inhibition of aflatoxins, diacetoxysciprenol and zearalenone production. *Folia Microbiology* **38**, 295-298.

Henriksen B, Langseth W, Sundheim L, 2000. Factors affecting Fusarium infection and mycotoxin content in Norwegian cereal grains. In: Proceedings of the 6th European Fusarium Seminar and Third COST 835 Workshop, 11th-16th September, Berlin, Germany, pp.108.

Hilton AJ, 1999. Mechanisms of resistance to Fusarium ear blight in winter wheat (*Triticum aestivum* L.). PhD Theses. Open University, UK.

Hilton AJ, Jenkinson P, Hollins TW and Parry DW, 1999. Relationship between cultivar height and severity of Fusarium ear blight in wheat. *Plant Pathology* **48**, 202-208.

Holbert JR, Trost JF, Hoffer GN, 1919. Wheat scab as affected by system of rotation. *Phytopathology* **9**, 45-47.

Homdork S, Fehrmann H, Beck R, 2000a. Influence of different storage conditions on the mycotoxin production and quality of *Fusarium*-infected wheat grain. *Journal of Phytopathology* **148**, 7-15.

Homdork S, Fehrmann H, Beck R, 2000b.Effects of field application of tebuconazole on yield, yield components and the mycotoxin content of *Fusarium*-infected wheat grain. *Journal of Phytopathology* **148**, 1-6.

Hsia YT, Hsio CP, Gao CX, 1958. Varietal resistance of wheat varieties to *Gibberella zeae* head blight. *Review of Applied Mycology* **37**, pp. 33.

Huang SX, 1992. Mycotoxicoses occurring in flooded areas and preventive measures against them. In: Chinese Ministry of Health (Ed.) Proceedings of Chinese Countermeasures for Anti-epidemic and Disaster Relief, Beijing: CMH, pp.45-49.

Humphreys J, Cooke BM, Storey T, 1995. Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter-sown wheat. *Plant Varieties and Seeds* **8**, 107-117.

Humphreys J, Cooke BM, Storey T, 1998. Effects of seed-borne *Microdochium nivale* on establishment and population density at harvest of winter-sown oats. *Plant Varieties and Seeds* **11**, 83-90.

Hussein SH and Brasel JM 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* **167**, 101-134.

Hutcheon JA and Jordan VWL 1990. Glasshouse evaluation of fungicides for control of *Fusarium* spp. on ears of winter wheat. *Annals of Applied Biology, Supp.***116**, 50-51.

Hutcheon JA and Jordan VWL 1992. Fungicide timing and performance for Fusarium control in wheat. Proceedings of the Brighton Crop Protection Conference-Pests and Disease **2**, 633-638.

Jacobsen BJ, 1977. Effect of fungicides on Septoria leaf and glume blotch, Fusarium scab, grain yield, and test weight of winter wheat. *Phytopathology* **67**, 1412-1414.

Jenkinson P, 1994. Epidemiology of *Fusarium* in Winter Wheat (*Triticum aestivum* L.). PhD Theses. Open University, UK.

Jenkinson P and Parry DW 1994. Isolation of *Fusarium* species from common broad-leaved weeds and their pathogenicity to winter wheat. *Mycological Research* **98**, 776-780.

Jenkinson P and Parry DW 1994. Splash dispersal of conidia of *Fusarium culmorum* and *Fusarium avenaceum*. *Mycological Research* **98**, 506-510.

Jennings P, Turner JA, Nicholson P, (2000) Overview of Fusarium ear blight in the UK – effect of fungicide treatment on disease control and mycotoxin production. In: Proceedings of The Brighton Crop Protection Conference – Pests and Diseases **2**, 707-712

Joffe A, 1978. *Fusarium poae* and *Fusarium sporotrichioides* as principal causes alimentary toxic aleukia. In: Wyllie, T.D., Morehouse, L.G. (Eds.) Handbook of micotoxins and Mycotoxicoses, Vol.3, New York: Marcel Dekker, p.21-86.

Jones RK, 2000. Assessments of Fusarium head blight of wheat and barley in response to fungicide treatment. *Plant Disease* **84**, 1021-1030.

Jones DR, Shearman V and Sylvester-Bradley R, (2001) Understanding the effects of new wheat fungicides on disease development, crop growth and yield. Home Grown Cereals Authority Report No. 261, September 2001.

Jordan VWL and Fielding EC 1988. *Fusarium spp.* on wheat. In: long Ashton Research Station Report for 1987. Long Ashton: Long Ashton Research Station Publication 23.

Kang Z and Buchenauer 2000. Ultrastructural and immunocytochemical investigation on pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. *Physiological and Molecular Plant Pathology* **57**, 255-268.

Kang Z, Huang L, Krieg U, Mauler-Machnik A, Buchenauer H, 2001. Effects of tebuconazole on morphology structure, cell wall components and trichothecene production of *Fusarium culmorum in vitro*. *Pest Management Science* **57**, 291-500.

Kang Z, Huang L, A, Buchenauer H, 2001. Ultrastructural and cytochemical studies of effects of the fungicide metconazole on *Fusarium culmorum in vitro*. *Journal of Plant Disease and Protection* **108**, 419-432.

Kang Z and Buchenauer 2002. Immunocytochemical localisation of β-1,3-glucanase and chitinase in *Fusarium culmorum*-infected wheat spikes. *Physiological and Molecular Plant Pathology* 60, 141-153.

Khan NI, Schisler DA, Boehm MJ, Slininger PJ, McCormick SP, 1999. Performance of selected antagonists of Fusarium head blight against a range of *Gibberella zeae* isolates. *Phytopathology* **89**, S39.

Kimura M, Anzai H, Yamaguchi I, 2001. Microbial toxins in plant-pathogen interactions: Biosynthesis, resistance mechanisms, and significance. *Journal of General and Applied Microbiology* 47, 149-160.

King RR, McQueen RE, Levesque D, Greenhalgh, R 1984. Transformation of deoxynivalenol (Vomitoxin) by rumen microorganisms. *Journal of Agriculture and Food and Chemistry* **32**, 1181-1183.

Koehler B, Dickson JG, Holbert JB, 1924. Wheat scab and corn rootrot caused by *Gibberella saubibetil* in relation to crop successions. *Journal of Agricultural Research* **27**, 861-883.

Khonga EB and Sutton JC 1986. Survival and inoculum production by *Gibberella zeae* in wheat and corn residues. *Canadaian Journal of Plant Pathology* **8**, p.531.

Krska R, Baumgartner S, Josephs R, 2001. The state-of-the art in the analysis of type-A and -B trichothecene mycotoxins in cereals. *Fresenius Journal of Analythical Chemistry* **371**, 285-299.

Kubena LF, Smith EE, Gentles A, Harvey RB, Edrington TS, Philips TD, Rottinghaus GE, 1994. Individual and combined toxicity of T-2 toxin and cyclopiazonic acid in broiler chicks. *Poultry Science* **73**, 1390-1397.

Lacey J, Bateman GL, Mirocha CJ, 1999. Effects of infection time and moisture on the development of ear blight and deoxynivalenol production by *Fusarium* spp. in wheat. *Annals of Applied Biology* **134**, 277-283.

Lees AK, Nicholson P, Rezanoor HN, Parry DW, 1995. Analysis of variation within *Microdochium nivale* from wheat: evidence for a distinct sub-group. *Mycological research* **99**, 103-109.

Li FQ, Luo XY, Yoshizawa T, 1999. Mycotoxins (trichothecenes, zearalenone and fumonisins) in cereals associated with human red intoxications stored since 1989 and 1991 in China. *Natural Toxins* **7**, 93-97.

Li FQ, Li YW, Luo XY, Yoshizawa T, 2002. *Fusarium* toxins in wheat from an area in Henan Province, PR China, with a previous human red mould intoxication episode. *Food Additives and Contaminants* **19**, 162-167.

Liggitt J, Jenkinson P, Parry DW, 1997. The role of saprophytic microflora in the development of Fusarium ear blight of winter wheat caused by *Fusarium culmorum*. *Crop Protection* **16**, 679-685.

Liggitt J, 1997. Studies on the chemical control of Fusarium ear blight of winter wheat (*Triticum aestivum* L.). PhD Thesis. Open University, UK.

Luo XY, Li, YW, Wen SF, Hu X, 1987. Determination of *Fusarium* mycotoxins in scabby wheat associated with human red-mold intoxication. *Hygiene Research* **16**, 33-37.

Luo XY, 1992. Food poisoning caused by *Fusarium* toxins in China. In: Proceedings of the Second Asian Conference on Food Safety International Life Science Institute. Bangkok, (pp.129-136).

MacInnes J and Fogelman R 1923. Wheat scab in Minnesota. *University of Minnesota Agricultural Experimental Station Technical Bulletin* **18**, pp.32.

Magan N and Lacey J 1984. Effect of water activity, temperature and substrate on interactions between field and storage fungi. *Transactions of the British Mycological Society* **82**, 83-93.

Mains EB, Vestal CM, Curtis PB, 1929. Scab of small grains and feed trouble in Indiana in 1928. Proceedings of the Indiana Academy of Science **39**, 101-110.

Martin RA and Johnston HW 1982. Effects and control of *Fusarium* diseases of cereal grains in the Atlantic Provinces. *Canadian Journal of Plant Pathology* **4**, 210-216.

Martin RA, MacLeod JA, Caldwell C, 1991. Influences of production inputs on incidence of infection by *Fusarium* species on cereal seed. *Plant Disease* **75**, 784-788.

Matthies A, Walker F, Buchenauer H, 1999. Interference of selected fungicides, plant growth retardants as well as piperonil butoxide and 1-aminobenzotriazole in trishcothecene production of *Fusarium graminearum* (strain 4528) *in vitro*. *Journal of Plant Disease and Protection* **106**, 198-212.

Matthies A and Buchenauer H 2000. Effect of tebuconazole (Folicur®) and prochloraz (Sportak®) treatments on Fusarium head scab development, yield and deoxynivalenol (DON) content in grains of wheat follosing artificial inoculation with *Fusarium culmorum*. *Journal of Plant Disease and Protection* **107**, 33-52.

Mauler-Machnik A and Zahn K 1994. Ear fusarioses in wheat-new findings on their epidemiology and control with Folicur® (tebuconazole). *Phlantzenschutz-Nachrichten Bayer* **47**, 129-155.

McMullen M and Nelson DR 1995.*Fusarium* head blight and *Septoria* diseases in wheat severe in North Dakota, 1994. (Abstr.) *Phytopathology* **85**, pp.1045.

McMullen M, Jones R, Galenberg D, 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Disease* **81**, 1340-1348.

McKay R, 1957. Ear blight, cereal scab, seedling blight of wheat and root rot of oats. In: Cereal diseases in Ireland, Arthur Guinness, Dublin, pp.74-83.

Mesterhazy A, 1984. *Fusarium* species of wheat in South Hungary, 1970-1983. *Cereal Research Communications* **12**, 167-170.

Mesterhazy A, 1995. Types and components of resistance to *Fusarium* head blight of wheat. *Plant Breeding* **114**, 377-386.

Mesterhazy A and Bartók T 1996. Control of Fusarium head blight of wheat by fungicide and its effect in the toxin contamination of the grains. *Pflanzenchutz-Nachrichten Bayer* **49**, 181-198.

Mesterhazy A, Bartók T, Mirocha CJ, Komoróczy R, 1999. Nature of wheat resistance to *Fusarium* head blight and the role of deoxynivalenol for breeding. *Plant Breeding* **118**, 97-
110.

Michail von S H, 1989. Effect of field sprays of wheat with fungicides and seed health in the Federal Republic of Germany. *Nachrichtenblat für Deutsche Pflantzenschutzd* **41**, 131-133.

Michuta-Grimm L and Foster RL 1989. Scab of wheat and barley in Southern Idaho and evaluation of seed treatments for eradication of *Fusarium spp. Plant Disease* **73**, 769-771.

Millar CS and Colhoun J 1969. Fusarium Diseases in Cereals. IV. Observation on *Fusarium nivale* on wheat. *Transaction of the British Mycological Society* **52**, 57-66.

Miller JK, Hacking A, Harrison J, Gross VJ, 1973. Stillbirths, neonatal mortality and small litters in pigs associated with the ingestion of *Fusarium* toxin by pregnant sows. *The Veterinary Record* **93**, 555-559.

Miller JD and Arnison PG 1986. Degradation of deoxynivalenol by suspension cultures of the *Fusarium* headblight resistant wheat cultivar Frontana. *Canadian Journal of Plant Pathology* **8**, 147-150.

Miller JD, Greenhalgh R, Wang YZ, Lu M, 1991. Trichothecene chemotypes of three *Fusarium* species. *Mycologia* **83**, 121-130.

Miller JD, Culley J, Fraser K, Hubbard S, Meloche F, Quellet T, Seaman WL, Seifer KA, Turkington K, Voldeng H, 1998. Effect of tillage practice on Fusarium head blight of wheat. *Canadian Journal of Plant Pathology* **20**, 95-103.

Milus EA and Parsons CE 1994. Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. *Plant Disease* **78**, 697-699.

Milus EA and Weight CT (1998) Efficacy of Quadris and Benlate Applications on Wheat Scab in Arcansas. In: Proceedings of National Fusarium Head Blight Forum, 26-27 Oct. 1998, East Lansing, MI, pp 51-52.

Mirocha CJ, Christensen CM, and Nelson GH, 1971. F-2 (zearalenone) estrogenic mycotoxin from *Fusarium*. In: S.Kadis, A.Cieger, and S.S. Ajl (Eds.) Microbial toxins. Vol 7, Algal and fungal toxins. Academic Press, New York.

Moschini RC, Pioli R, Carmona M, Sacchi O, 2001. Empirical predictions of wheat Head blight in the Northern Argentinean Pampas region. *Crop Science* **41**, 1541-1545.

Moss M O and Frank JM 1985. Influence of the fungicide tridemorph on T-2 tohin production by *Fusarium sporotrichoides*. *Transactions of the British Mycological Society* **84**, 585-590.

Morgain D, Couture L, Dubuc JP, Comeau A, 1997. Occurrence of the orange wheat blossom midge [Diptrea : Cecidomyiidae] in Québec and its incidence on wheat grain microflora. *Phytoprotection* **78**, 17-22.

Morgain D, Couture L, Comeau A, 2000. Natural occurrence of Fusarium graminearum and adult wheat midge and transmission to wheat spikes. *Cereal Research Communications* **28**, 173-179.

Munteanu I, Muresan T, Tataru V, 1972. *Fusarium* wilt in wheat and integrated disease control in Romania. *Acta Agronomica Academiae Scientiarum Hungaricae* **21**, 17-29.

Narziss L, Back W, Reinchneder E, Simon A, Grandl R, 1990. Investigation into the gushing problem. *Monatsschrift für Brauwissenschaft* **43**, 296-305.

Nelson R, 1929. Wheat scab damages Michigan grain crops, Favourable weather conditions increase danger of damage by this disease. *Michigan Quarterly Bulletin* **12**, 8-15.

Nierenberg HI, 1981. A simplified method for identifying *Fusarium* spp. occurring on wheat. *Canadian Journal of Botany* **59**, 1599-1609.

Nightingale MJ, Marchylo JE, Clear RM, Dexter RM, Preston KR, 1999. Fusarium Head Blight: Effect of fungal proteases on wheat storage proteins. *Cereal Chemistry* **76**, 150-158.

Odhav B and Naiker V 2002. Mycotoxins in South African traditionally brewed beers. *Food Additives and Contaminants* **19**, 55-61.

Obst A, Lepschy-von Gleissenthall J, Beck R, 1997. On the aetiology of Fusarium head blight of wheat in South Germany- Preceding crops, weather conditions for inoculum production and head infection, proneness of the crop to infection and mycotoxin production. *Cereal Research Communications* **25**, 699-703.

Obst A, Lepschy J, Beck, R, Bauer G, 2000. Risk factors for the head infection of wheat and the production of deoxynivalenol by *Fusarium graminearum*, derived from the Fusarium monitoring in Bavaria. In: Proceedings of the 6th European Fusarium Seminar and Third COST 835 Workshop, 11th-16th September, Berlin, Germany, pp. 110.

Parry DW, 1990. The incidence of *Fusarium* spp in stem bases of selected crops of winter wheat in the Midlands, UK. *Plant Pathology* **39**, 619-622.

Parry DW, Jenkinson P, McLeod L, 1995a. Fusarium ear blight (scab) in small grain cereals-a review. *Plant Pathology* **44**, 207-238.

Parry DW, Rezanoor HN, Pettit TR, Hare MC, Nicholson P, 1995b. Analysis of *Microdochium nivale* isolates from wheat in the UK during 1993. *Annals of Applied Biology* **126**, 449-455.

Pasquini M, Cardone AM, Casini F, Casulli F, Chierico M, Contoli S, Converso R, 2001. Le principali malattie del frumento. *L'informatore agrario* **1**, 379-380.

Paulitz TC, 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. *Plant Disease* **80**, 674-678.

Pettitt TR, Parry DW, Polley RW, 1993. Improved estimation of the incidence of *Microdochium nivale* in winter wheat stems in England and Wales, during 1992, by use of benomyl agar. *Mycological Research* **97**, 1172-1174.

Placinta CM, Macdonald AMC, D'Mello JPF, Harling R, 1996.The influence of carbendazim on mycotoxin production in *Fusarium sporotrichioides*. In. Proceedings of the Brighton Crop Protection Conference, BCPC, Farnham, UK, pp. 415-416.

Placinta CM, D'Mello JPF, Macdonald AMC, 1999. A review of worldwide contamination of cereal grains and animal feed with *Fusarium* mycotoxins. *Animal Feed Science and Technology* **78**, 21-37.

Polley RW and Thomas MR, 1991. Surveys on of diseases of winter wheat in England and Wales, 1976-1988. *Annals of Applied Biology* **119**, 1-20.

Polley RW, Turner JA, Cockerell V, Robb J, Scudamore KA, Sanders MF, Magan N, 1991. Survey of *Fusarium* species infecting winter wheat in England, Wales and Scotland, 1989-1990. Home Grown Cereal Authority Project Report 39, London.

Pricket AJ, MacDonald S and Wildey KB, (2000) Survey of mycotoxins in stored grain from the 1999 harvest in the UK. HGCA Project Report No 230. HGCA, London

Prom LK, Horsley RD, Steffenson BJ, Schwarz PB, 1999. Development of Fusarium head blight and accumulation of deoxynivalenol in barley sampled at different growth Stages. *Journal of the American Society of Brewing Chemists* **57**, 60-63.

Rapilly F, Lamaire JM, Cassini R, 1973. Les Fusarioses. In: Les Maladies des Céréales. Paris: INRA/ITFC, 119-138.

Reinecke P, Duben J, Fehrman H, 1979. Antagonism between fungi of the foot rot complex of cereals. In: Schippers, B. and Gams, W. (Eds.) Soil-Borne Plant Pathogens, Academic Press, London, pp. 327-336.

Rennie WJ, Richardson MJ, Noble M, 1983. Seed-borne pathogens and the production of quality seed in Scotland. *Seed Science and Technology* **11**, 1115-1127.

Rintelen J, 1992. Fusaria in oat seeds – in a survey in Bavaria1985-1989. *Gesunde Pflanzen* **44**, 388-392.

Rotter BA, Thompson BK, Lessard M, 1995. Effects of deoxynivalenol-contaminated diet on performance and blood parameters in growing swine. *Canadian Journal of Animal Science* **75**, 297-302.

Rotter BA, Prelusky DB, Pestka JJ, 1996. Toxicology of deoxynivalenol (vomitoxin). *Journal of Toxicology and Environmental Health* **48**, 1-34.

Ruy D and Bullerman LB, 1999. Effect of cycling temperature on the production of deoxynivalenol and zearalenone by *Fusarium graminearum* NRRL 5883. *Journal of Food Protection* **62**, 1451-1455.

Salas B, Steffenson BJ, Casper HH, Tacke B, Prom LK, Fetch TG, Schwarz PB, 1999. *Fusariuim* species pathogenic to barley and their associated mycotoxins. *Plant Disesase* **83**, 667-674.

Sayler T, 1998. Study: $ 2.6 billion, 501 million bushels lost to scab 1991-96. *Prairie Grains* **11**

Schipilova NP and Gagkaeva TY 1997. The forms of manifestation of *Fusarium* head blight on the seed and heads of cereal crops. *Cereal Research Communications* **25**, 815-816.

Schwarz PB, Schwarz JG, Zhou A, Prom LK, Steffenson BJ 2001. Effect of *Fusarium graminearum* and *F. poae* infection on Barley and Malt Quality. *Monatsschrift für Brauwissenschaft* **54**, 55-63.

Scott PM, Lau PY, Kanhere SR, 1981. Gas chromatography with electron capture and mass spectrometric detection of deoxynivalenol in wheat and other grains. *Journal of Assosiacion of Analithical Chemistry* **64**, 1364-1371.

Schroeder HW and Christensen JJ 1963. Factors affecting the resistance of wheat to scab caused by *Gibberella zeae*. *Phytopathology* **53**, 831-838.

Simpson DR, Weston GE, Turner JA, Jennings P, Nicholson P, 2001. Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European Journal of Plant Pathology* **107**, 421-431.

Sinha RC and Savard ME 1997. Concentration of deoxynivalenol in single kernels and various tissues of wheat heads. *Canadian journal of Plant Pathology* **19**, 8-12.

Siranidou E and Buchenauer H 2001. Chemical control of Fusarium head blight on wheat. *Journal of Plant Disease and Protection* **108**, 231-243.

Snijders CHA, 1990. Systemic fungal growth of *Fusarium culmorum* in stems of winter wheat. *Journal of Phytopathology* **129**, 113-140.

Snijders CHA, 1994. Breeding for resistance to *Fusarium* in wheat and maize. In: Miller JD, Trenholm HL (Eds.) Mycotoxins in Grain-Compounds Other Than Aflatoxins (pp. 37-58), Eagan Press St.Paul, Minnesota.

PAGE

NUMBERING

AS ORIGINAL

APPENDICES

Appendix 1. Ingredients used for the preparation of Low Nutrition Agar (SNA) (Nierenberg, 1981)

1.0g KH_2PO_4

1.0g KNO_3

0.5g $MgSO_4.7H_2O$

0.5g KCl

0.2g Glucose

0.2g Sucrose

20g Agar

1l distilled water

Appendix 2. Husbandry practices adopted during field trial 1 (1998/1999).

Date	Operation
26/10/98	Seed cv Equinox treated with Panoctine (guazatine) drilled at 375 seeds m^{-2} and ring rolled
23/03/99	Spread Nitram (35.4 % N) @ 42kgN ha^{-1}
24/03/99	Sprayed herbicides – Panter (diflufenican + isoproturon) 0.6 l ha^{-1} + Isogard (isoproturon) 2 l ha^{-1} + Ally (metsulfuron-methiyl) 0.015 kg ha^{-1}
06/04/99	Spread Nitram (35.4 %) @ 103.5 kg N ha^{-1}
28/04/99	Sprayed fungicides – Rover 500 (chlorothalonil) 1.0 l ha^{-1}
28/04/99	Sprayed plant growth regulator – New 5C Cycocel (chlormequat + choline chloride) 2.5 l ha^{-1}
26/05/99	Sprayed fungicides – Rover 500 (chlorothalonil) 2.0 l ha^{-1} + Opus (epoxiconazole) 0.7 l ha^{-1} + Fortress (quinoxyfen) 0.2l ha^{-1}
26/05/99	Sprayed plant growth regulator- Terpal (2-chloroethylphosphonic acid + mepiquat chloride) 2.0 l ha^{-1}
31/08/99	Plots harvested

Appendix 3. Husbandry practices adopted during field trial 2 (1999/2000).

Date	Operation
22/10/1999	Seed cv Equinox treated with "Panoctine"(guazatine) drilled at 375 seeds m^{-2} and ring rolled
11/03/00	Sprayed fungicide - Fortress (quinoxyfen) 0.3 l ha^{-1}
17/03/00	Spread granular sulphur 75 kg ha^{-1}
10/04/00	Spread Nitram (34.5% N) @ 100 kg N ha^{-1}
09/05/00	Sprayed herbicides - Starane 2 (fluroxypyr)1.0 l ha^{-1} + Ally (metsulfuron-methiyl) 0.015 kg ha^{-1}
09/05/00	Sprayed - Manifol (manganese) 0.5 l ha^{-1}
18/05/00	Sprayed plant growth regulator- Terpal (2-chloroethylphosphonic acid + mepiquat chloride) 0.75 l ha^{-1}
20/05/00	Sprayed fungicide - Opus (epoxiconazole) 0.7 l ha^{-1}
24/05/00	Spread Nitram (34.5% N) 40kg N ha^{-1}
31/08/00	Plots harvested

Appendix 4. Husbandry practices adopted during field trial 3 (2000/2001).

Date	Operation
06/03/01	Seed cv Cadenza treated with "Panoctine"(guazatine) drilled at 375 seeds m^{-2} and ring rolled
10/05/01	Sprayed insecticide - Cyperkill (cypermethrin) 0.25l ha^{-1}
10/05/01	Sprayed - Manifol (manganese) 1l ha^{-1}
16/05/01	Spread Nitram (34.5% N) @ 100 kg N ha^{-1}
22/05/01	Starane 0.5 l ha^{-1} + Ally 0.20 kg ha^{-1} + Manganese 0.5l ha^{-1}
22/05/01	Sprayed - Manifol (manganese) 1l ha^{-1}
25/05/01	Sprayed fungicides - Landmark (epoxiconazole + kresoxim-methyl) 0.5l ha^{-1} + Unix (cyprodinil) 0.4kg ha^{-1}
30/05/01	Spread Nitram (34.5% N) 60kg N ha^{-1}
08/06/01	Sprayed fungicides - Landmark (epoxiconazole + kresoxim-methyl) 1.3l ha^{-1}
28/08/01	Plots harvested

Appendix 5. The effect of fungicides applied at GS 59 to plots of winter wheat (cv Equinox) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the incidence of FHB recorded 21 and 28 days post-inoculation and sooty moulds in a field trial 1 (1998/1999).

Treatment	Fungicides	Rates (g a.i. ha^{-1})	Arcsine % heads infected GS 75	Arcsine % heads infected GS 85	Sooty mould index
1	Unsprayed control		86.42 (99.61)	90.00 (100.0)	8.83
2	metconazole	90	84.02 (98.91)	82.75 (98.40)	4.83
3	azoxystrobin	250	73.01 (91.46)	81.45 (97.78)	1.50
4	tebuconazole	250	80.55 (97.30)	79.33 (96.57)	3.83
5	metconazole	45	84.81 (99.18)	80.92 (97.50)	6.00
6	azoxystrobin	125	77.65 (95.54)	78.93 (96.31)	4.64
7	tebuconazole	125	79.93 (96.94)	85.53 (99.39)	6.00
8	metconazole azoxystrobin	45 125	60.48 (75.72)	65.19 (82.39)	3.17
LSD (5%) df=35			6.7 (P<0.001) CV= 7.3 %	5.51 (P<0.001) CV= 5.8 %	1.21 (P<0.001) CV=21.3 %

Appendix 6. The effect of fungicides applied at different timing to plots of winter wheat (cv Cadenza) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the incidence of FHB and sooty mould in field trial 3 (2000/2001).

Treatment	Fungicide	Rates (g a. i. ha^{-1})	Time of application	Arcsine % heads infected GS 75	Arcsine % heads infected GS 85	Sooty mould index
1	Control			38.21 (38.25)	38.72 (39.12)	6.50
2	metconazole	45	5 dbi*	29.13 (23.69)	26.54 (19.96)	3.25
3	tebuconazole	125	5 dbi	28.13 (22.22)	26.40 (19.77)	4.25
4	azoxystrobin	125	5 dbi	30.22 (25.33)	29.54 (24.30)	2.25
5	metconazole+ azoxystrobin	45+ 125	5 dbi	28.14 (22.24)	25.15 (18.06)	3.25
6	tebuconazole+ azoxystrobin	45 + 125	5 dbi	25.36 (18.34)	25.89 (19.06)	4.25
7	metconazole	45	2 dbi	22.24 (14.32)	25.02 (17.88)	4.50
8	tebuconazole	125	2 dbi	28.84 (23.26)	25.22 (18.15)	4.50
9	azoxystrobin	125	2 dbi	31.85 (27.84)	28.86 (23.29)	2.50
10	metconazole+ azoxystrobin	45+ 125	2 dbi	18.81 (10.39)	21.47 (13.39)	2.50
11	tebuconazole+ azoxystrobin	45+ 125	2 dbi	21.91 (13.92)	23.16 (15.46)	2.00
12	metconazole	45	2 dai**	17.93 (09.47)	25.30 (18.26)	1.75
13	tebuconazole	125	2 dai	27.33 (21.07)	24.08 (16.64)	3.50
14	azoxystrobin	125	2 dai	26.79 (20.31)	29.93 (24.89)	3.25
15	metconazole+ azoxystrobin	45+ 125	2 dai	22.35 (14.46)	23.32 (15.67)	2.75
16	tebuconazole+ azoxystrobin	45+ 125	2 dai	19.25 (10.85)	24.11 (16.68)	3.00
17	metconazole	45	5 dai	28.52 (22.79)	27.63 (21.50)	3.25
18	tebuconazole	125	5 dai	26.90 (20.46)	24.47 (17.15)	2.75
19	azoxystrobin	125	5 dai	31.17 (26.78)	31.27 (26.94)	3.75
20	metconazole+ azoxystrobin	45+ 125	5 dai	26.55 (19.97)	22.44 (14.57)	3.00
21	tebuconazole+ azoxystrobin	45+ 125	5 dai	23.02 (15.29)	27.39 (21.16)	2.25
LSD (5%)				5.23	3.23	1.45
Fungicide				(P<0.001) CV=17.8 %	(P<0.001) CV=10.09 %	(P>0.05) CV= 40.7 %
Time				5.12 (P<0.05) CV=17.8 %	3.16 (P>0.05) CV=10.09 %	1.42 (P>0.05) CV= 40.7 %
Fungicide*time				6.61 (P>0.05) CV=17.8 %	4.08 (P>0.05) CV=10.09 %	1.84 (P>0.05) CV= 40.7 %

* days before inoculation
** days after inoculation

Appendix 7. The effect of fungicides applied at GS 59 to plots of winter wheat (cv Equinox) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the % incidence of *F. culmorum*, *F. graminearum* and *M. nivale* in wheat grain harvested in field trial 1 (1998/1999).

Treatment	Fungicide	Rates (g a.i. ha^{-1})	% grain infection *F. culmorum*	% grain infection *F. graminearum*	% grain infection *M. nivale*
1	Unsprayed control		72.33	26.67	21.17
2	metconazole	90	67.00	33.00	31.67
3	azoxystrobin	250	69.67	30.33	4.17
4	tebuconazole	250	75.83	24.17	28.83
5	metconazole	45	72.83	27.17	28.67
6	azoxystrobin	125	74.83	25.17	5.67
7	tebuconazole	125	72.00	28.00	30.17
8	metconazole+ azoxystrobin	45+ 125	70.33	29.67	6.83
LSD (5%)			7.45 (P>0.05) CV= 8.8 %	7.53 (P>0.05) CV= 8.7 %	6.04 (P<0.001) CV= 26.3 %

Appendix 8. The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize at GS 23-25 on the incidence of *Fusarium* spp. and *M. nivale* on the leaves of wheat collected at GS 37 in a field trial 2 (1999/2000).

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of application	Arcsine % leaves infected GS 37 *Fusarium* spp.	Arcsine % leaves infected GS 37 *M. nivale*
1	Unsprayed control			38.9 (39.4)	39.1 (39.7)
2	metconazole	90	31+39	30.1 (25.1)	40.1 (41.4)
3	metconazole	90	39+59	27.7 (21.60)	36.0 (34.5)
4	azoxystrobin	250	31+39	36.1 (34.71)	29.9 (24.8)
5	azoxystrobin	250	39+59	37.7 (37.3)	31.0 (26.5)
6	metconazole+ azoxystrobin	45+ 125	31+39	30.9 (26.3)	31.4 (27.1)
7	metconazole+ azoxystrobin	45+ 125	39+59	28.2 (22.3)	19.6 (11.2)
8	fluquinconazole	100	31+39	38.9 (39.4)	34.7 (32.4)
9	fluquinconazole	100	39+59	37.7 (37.3)	28.2 (22.3)
df 35					
LSD (5%)	Fungicide			9.87 (P<0.05) CV = 23.0 %	10.84 (P>0.05) CV = 26.6 %
LSD (5%)	Time			9.01 (P>0.05) CV = 23.0 %	9.90 (P>0.05) CV = 26.6 %
LSD (5%)	Fungicide*time			11.4 (P>0.05) CV = 23.0 %	12.52 (P>0.05) CV = 26.6 %

Appendix 9. The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize at GS 23-25 on the incidence of *Fusarium* spp. and *M. nivale* on the leaves of wheat collected at GS 51 in a field trial 2 (1999/2000).

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of application	Arcsine % leaves infected GS 51 *Fusarium* sp.	Arcsine % leaves infected GS 51 *M. nivale*
1	Unsprayed control			30.9 (26.3)	38.9 (39.4)
2	metconazole	90	31+39	30.6 (25.9)	34.6 (32.2)
3	metconazole	90	39+59	22.7 (14.8)	30.8 (26.2)
4	azoxystrobin	250	31+39	24.8 (17.5)	30.9 (26.3)
5	azoxystrobin	250	39+59	30.0 (25.0)	27.9 (21.8)
6	metconazole+ azoxystrobin	45+ 125	31+39	09.3 (02.6)	34.0 (31.2)
7	metconazole+ azoxystrobin	45+ 125	39+59	17.5 (09.04)	28.2 (22.35)
8	fluquinconazole	100	31+39	32.9 (29.5)	39.2 (39.9)
9	fluquinconazole	100	39+59	30.2 (25.3)	34.0 (31.2)
LSD (5%)	Fungicide			18.62 (P>0.05) CV = 58.0 %	11.32 (P>0.05) CV = 27.0 %
LSD (5%)	Time			17.00 (P>0.05) CV = 58.0 %	10.33 (P>0.05) CV = 27.0 %
LSD (5%)	Fungicide*time			21.5 (P>0.05) CV = 58.0 %	13.07 (P>0.05) CV = 27.0 %

Appendix 10. The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize at GS 23-25 on the incidence of *Fusarium* spp. and *M. nivale* on the leaves of wheat collected at GS 65 and sooty moulds in a field trial 1 (1999/2000).

Treatment	Fungicide	Rates (g a. i. ha^{-1})	GS of application	Arcsine % leaves infected GS 65 *Fusarium* sp.	Arcsine % leaves infected GS 65 *M. nivale*	Sooty moulds index
1	Unsprayed control			39.1 (39.7)	43.6 (47.5)	7.75
2	metconazole	90	31+39	34.5 (32.0)	42.0 (44.7)	6.00
3	metconazole	90	39+59	29.4 (24.5)	39.1 (39.7)	4.00
4	azoxystrobin	250	31+39	31.4 (27.1)	36.2 (34.8)	8.00
5	azoxystrobin	250	39+59	32.8 (29.3)	27.3 (21.0)	5.50
6	metconazole	45	31+39	27.9 (21.8)	41.9 (44.6)	8.25
	azoxystrobin	125				
7	metconazole	45	39+59	20.5 (12.2)	31.0 (26.5)	2.75
	azoxystrobin	125				
8	fluquinconazole	100	31+39	36.2 (34.8)	37.4 (36.8)	7.50
9	fluquinconazole	100	39+59	37.4 (36.8)	40.2 (41.6)	5.75
LSD (5%)	Fungicide			9.59 (P<0.05) CV = 23.6 %	12.88 (P>0.05) CV = 27.1%	1.40 (P=0.01) CV= 18.0 %
LSD (5%)	Time			8.75 (P>0.05) CV = 23.6 %	11.75 (P>0.05) CV = 27.1%	1.28 (P<0.001) CV= 18.0 %
LSD (5%)	Fungicide*time			11.07 (P>0.05) CV = 23.6 %	14.87 (P>0.05) CV = 27.1%	1.62 (P<0.01) CV= 18.0 %

Appendix 11. The effect of fungicides applied at GS 59 to plots of winter wheat (cv Equinox) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml^{-1} of water) on the yield and its parameters in a field trial 1 (1998/1999).

Treatment	Fungicide	Rates (g a.i. ha^{-1})	Grain yield t ha^{-1}	1000 grain weight (g)	Specific grain weight (kg hl^{-1})
1	Unsprayed control		3.730	45.66	57.25
2	metconazole	90	4.468	51.78	62.97
3	azoxystrobin	250	4.319	47.33	58.33
4	tebuconazole	250	4.310	53.47	68.83
5	metconazole	45	4.229	50.87	61.83
6	azoxystrobin	125	4.312	49.77	59.72
7	tebuconazole	125	4.520	51.82	62.30
8	metconazole+	45+	4.741	53.14	62.92
	azoxystrobin	125			
LSD (5%)			0.302 (P<0.001) CV= 6.0 %	2.25 (P<0.001) CV= 3.8 %	1.49 (P<0.001) CV= 2.1 %

Appendix 12. The effect of fungicides applied at various growth stages to plots of winter wheat (cv Equinox) inoculated with shredded maize at GS 23-25 on the on the yield and its parameters in a field trial 1 (1999/2000).

Treatment	Fungicide	Rates (g. a. i. ha)$^{-1}$	GS of Application	Grain yield t ha^{-1}	1000 grain weight (g)	Specific grain weight (kg hl^{-1})
1	Unsprayed control			4.06	31.99	47.83
2	metconazole	90	31+39	4.20	35.43	51.18
3	metconazole	90	39+59	5.09	42.44	60.20
4	azoxystrobin	250	31+39	4.66	36.63	54.00
5	azoxystrobin	250	39+59	4.70	39.27	53.10
6	metconazole	45	31+39	4.77	38.09	55.65
	azoxystrobin	125				
7	metconazole	45	39+59	5.38	43.58	58.30
	azoxystrobin	125				
8	fluquinconazole	100	31+39	4.43	34.97	54.00
9	fluquinconazole	100	39+59	4.22	37.46	52.88
LSD (5%)	Fungicide			0.33 (P<0.001) CV = 5.7 %	4.33 (P>0.05) CV = 9.1 %	6.10 (P>0.05) CV = 8.9 %
LSD (5%)	Time			0.30 (P = 0.001) CV = 5.7 %	3.95 (P=0.01) CV = 9.1 %	5.57 (P>0.05) CV = 8.9 %
LSD (5%)	Fungicide*time			0.38 (P = 0.001) CV = 5.7 %	5.00 (P>0.05) CV = 9.1 %	7.05 (P>0.05) CV = 8.9 %

Appendix 13. The effect of fungicides applied at different timing to plots of winter wheat (cv Cadenza) inoculated at GS 65 with a conidial suspension of *F. culmorum*, *F. graminearum* and *M. nivale* (10^5 spores per ml of water) on the yield, 1000 grain weight and specific grain weight in field trial 2 (2000/2001).

Treatment No.	Fungicide	Rates (g a. i. ha^{-1})	Time of application	Grain yield t ha^{-1}	1000 grain weight (g)	Specific grain weight (kg hl^{-1})
1	Unsprayed control			9.343	49.75	74.700
2	metconazole	45	5 dbi*	9.650	49.15	74.450
3	tebuconazole	125	5 dbi	9.664	50.65	74.350
4	azoxystrobin	125	5 dbi	9.604	51.25	75.050
5	metconazole+ azoxystrobin	45+ 125	5 dbi	9.777	51.27	75.100
6	tebuconazole+ azoxystrobin	45+ 125	5 dbi	9.562	51.05	74.850
7	metconazole	45	2 dbi	9.990	49.17	74.850
8	tebuconazole	125	2 dbi	9.419	50.45	74.350
9	azoxystrobin	125	2 dbi	9.468	49.38	74.850
10	metconazole+ azoxystrobin	45+ 125	2 dbi	9.861	49.25	75.600
11	tebuconazole+ azoxystrobin	45+ 125	2 dbi	9.837	44.90	74.950
12	metconazole	45	2 dai**	9.842	50.45	74.650
13	tebuconazole	125	2 dai	9.551	51.17	74.900
14	azoxystrobin	125	2 dai	9.362	51.85	75.250
15	metconazole+ azoxystrobin	45+ 125	2 dai	9.800	50.75	75.050
16	tebuconazole+ azoxystrobin	45+ 125	2 dai	10.057	51.42	75.150
17	metconazole	45	5 dai	9.815	50.85	74.650
18	tebuconazole	125	5 dai	9.743	50.67	75.400
19	azoxystrobin	125	5 dai	9.287	51.47	74.525
20	metconazole+ azoxystrobin	45+ 125	5 dai	9.851	51.60	74.450
21	tebuconazole+ azoxystrobin	45+ 125	5 dai	9.911	51.30	75.875
LSD (5%) Fungicide				0.316 (P<0.001) CV=2.9 %	1.65 (P>0.05) CV=5.9 %	0.933 (P>0.05) CV=1.1 %
Time				0.309 (P>0.05) CV=2.9 %	3.24 (P<0.05) CV=5.9 %	0.914 (P>0.05) CV=1.1 %
Fungicide*time				0.399 (P>0.05) CV=2.9 %	4.19 (P>0.05) CV=5.9 %	1.180 (P>0.05) CV=1.1 %

* days before inoculation;
** days after inoculation

Appendix 14 Effect of artificial inoculation on ears of winter wheat (cv Cadenza) with *Microdochium nivale* at GS 57 or 65+ (24 hours after inoculation with *F. culmorum*) alone or in combination with metconazole or azoxystrobin applied at GS 59 on the *M.nivale* DNA quantity in grain. Numbers in parentheses are back-transformed means

Time of inoculation or fungicide application				Log_{10} *M.nivale* DNA (pg ng^{-1} total DNA)
GS 57	GS 59	GS 65	GS 65+	
M. nivale		*F. culmorum*		1.32 (20.89)
		F. culmorum	*M. nivale*	0.73 (05.37)
M. nivale	metconazole	*F. culmorum*		1.30 (19.95)
M. nivale	azoxystrobin	*F. culmorum*		0.22 (01.65)
	metconazole	*F. culmorum*	*M. nivale*	0.73 (05.37)
	azoxystrobin	*F. culmorum*	*M. nivale*	0.26 (01.81)
LSD				0.54 (P<0.001)